

2/55

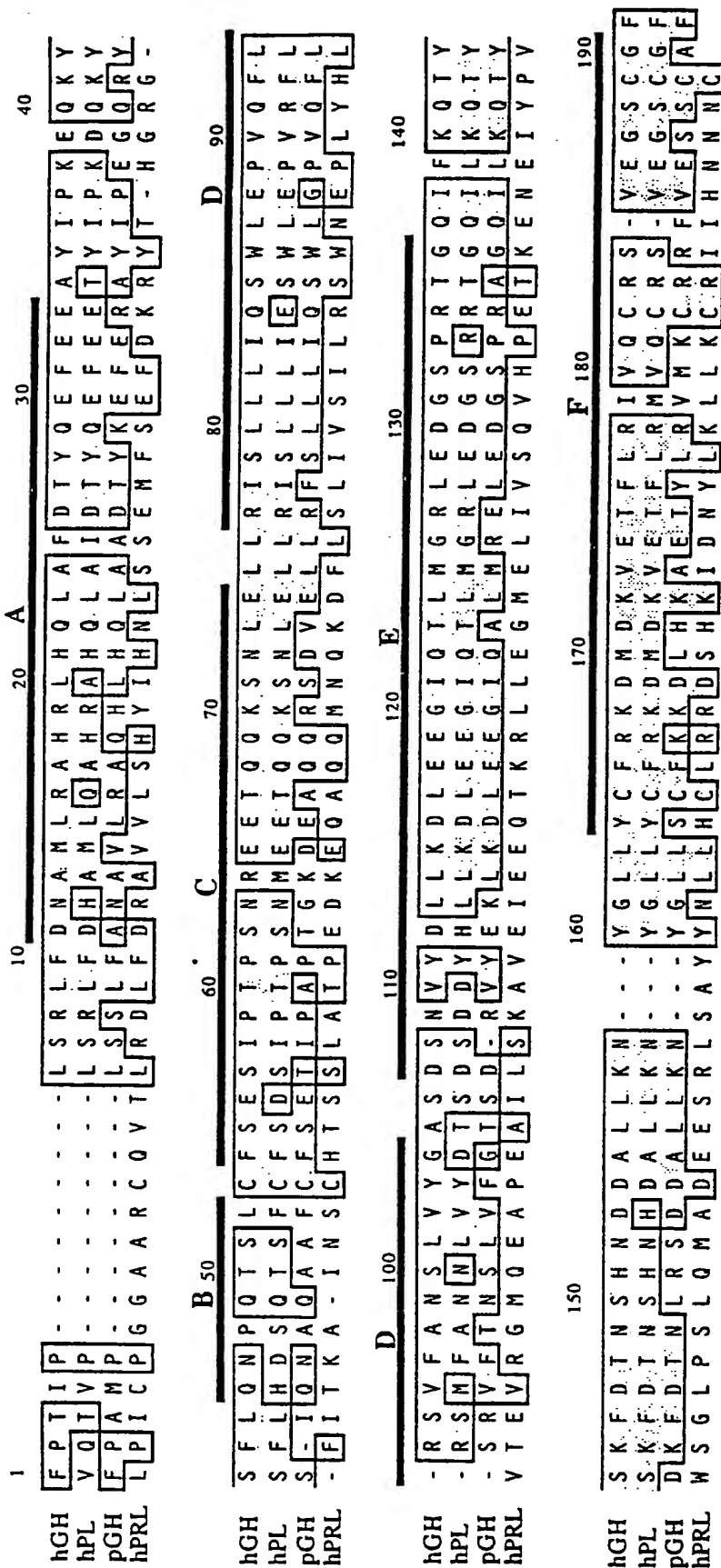
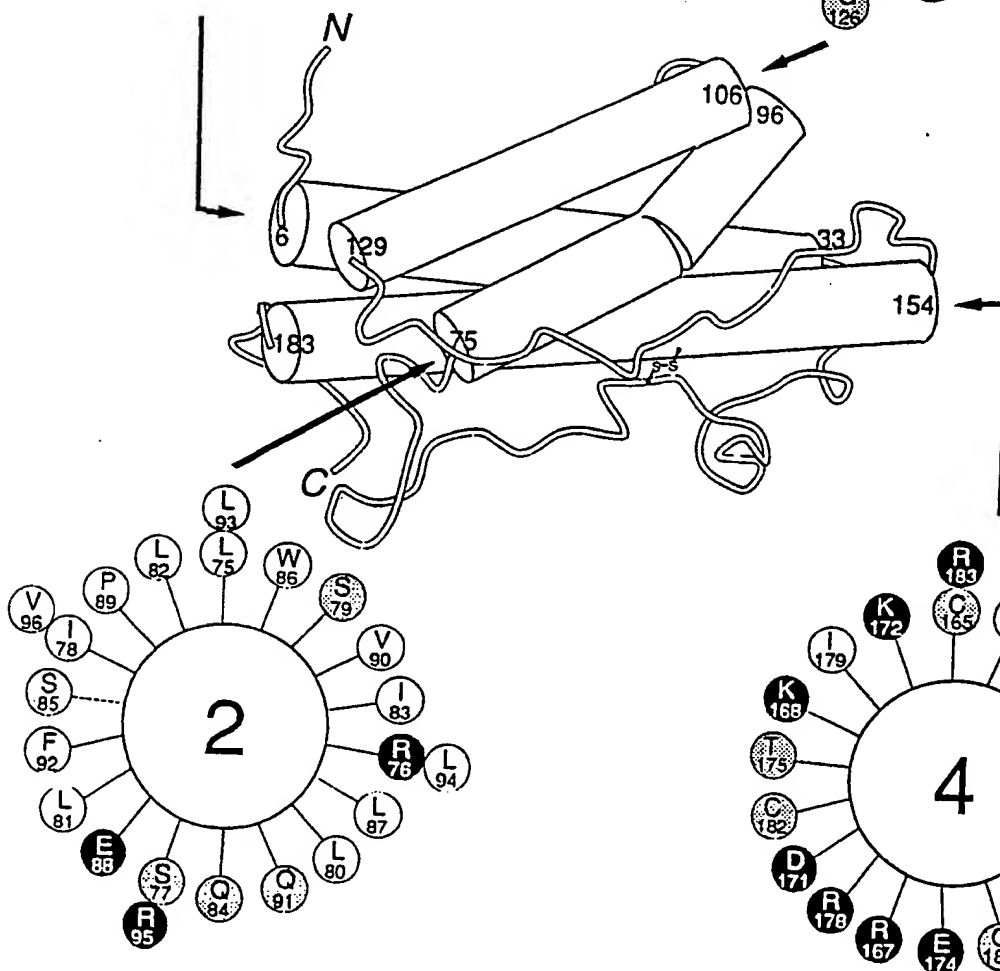
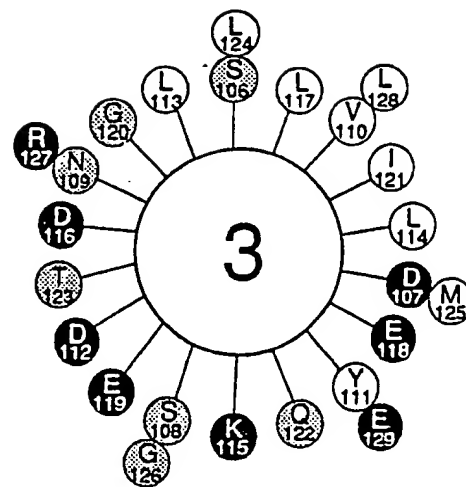


FIG.-2



**FIG.—3**

**SUBSTITUTE SHEET**

4/55

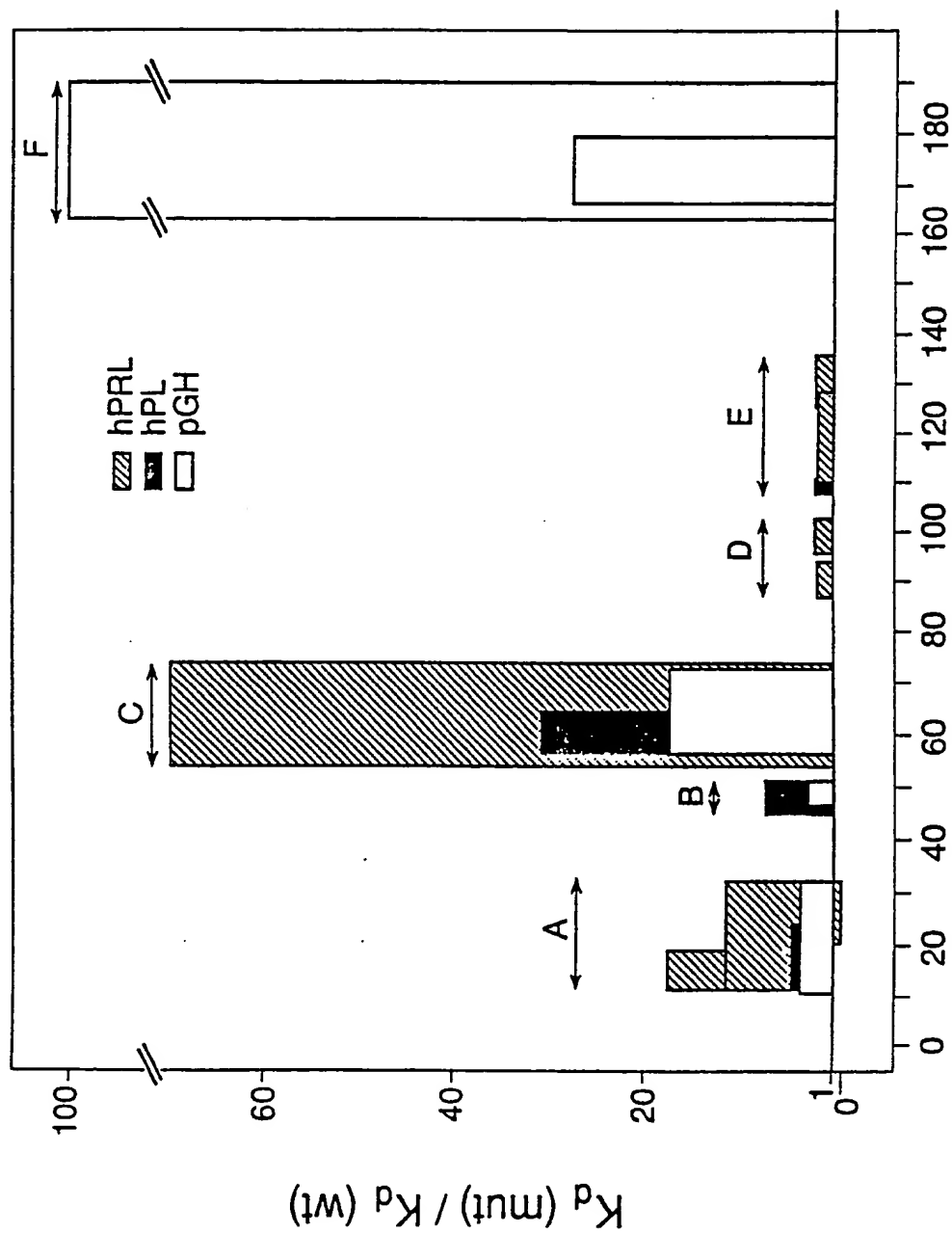


FIG.—4

Residue number in hGH

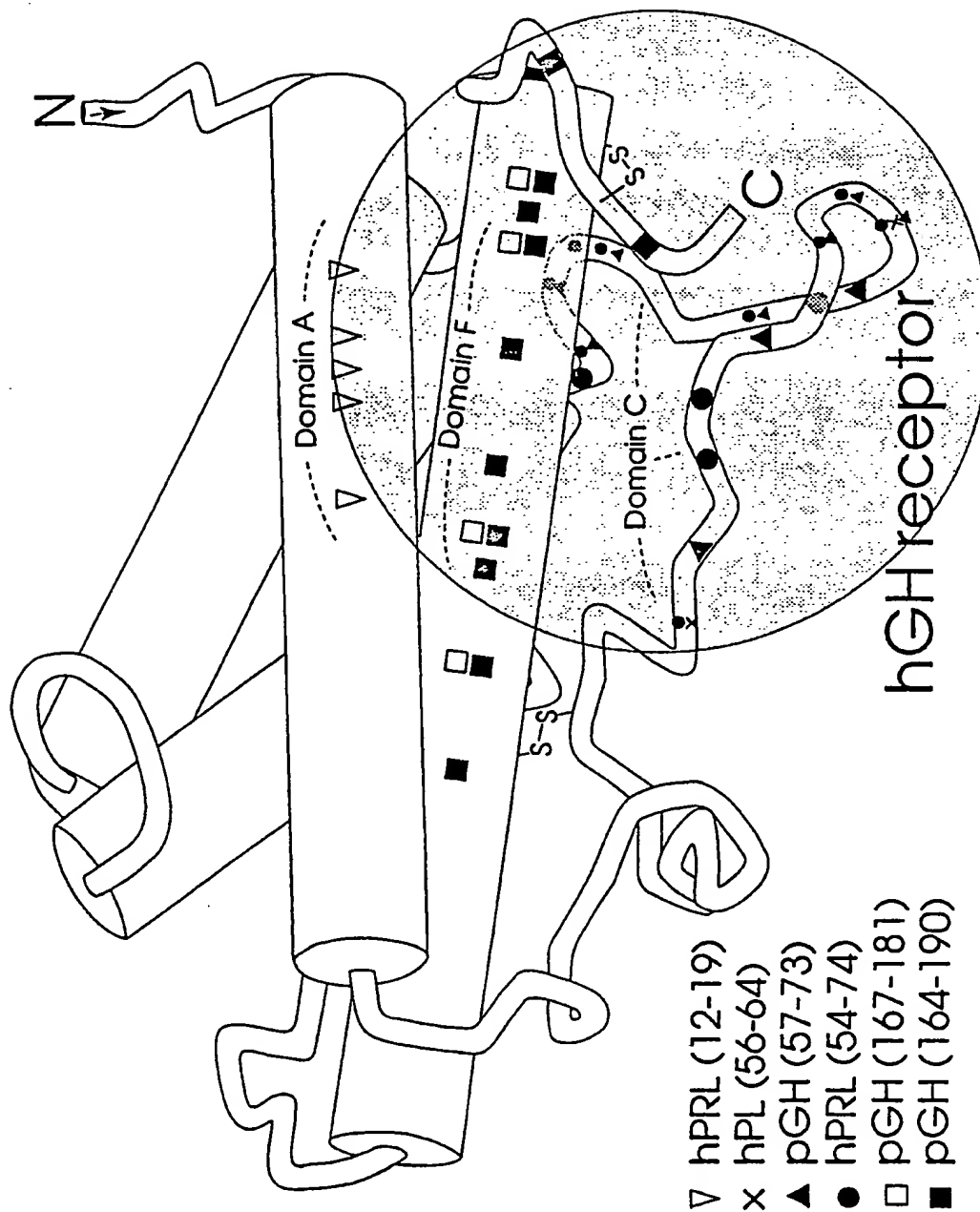
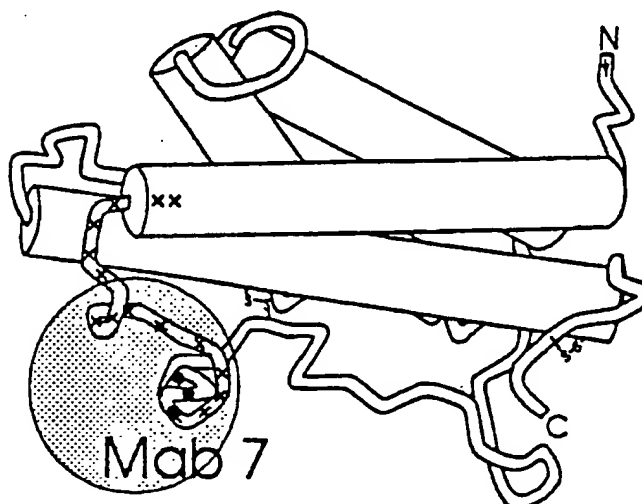
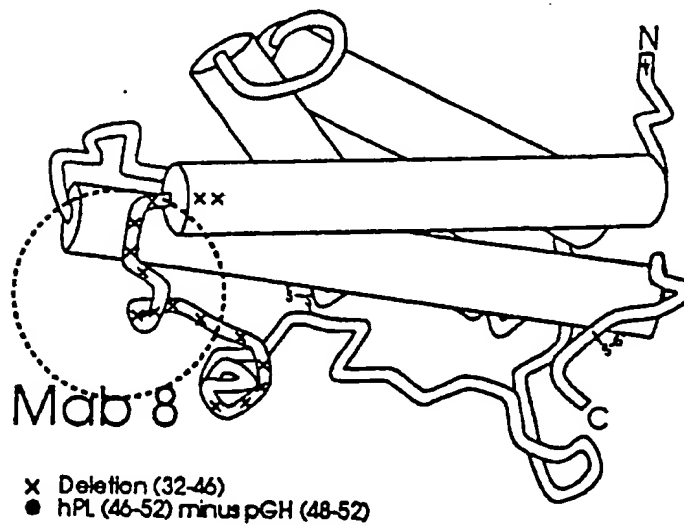
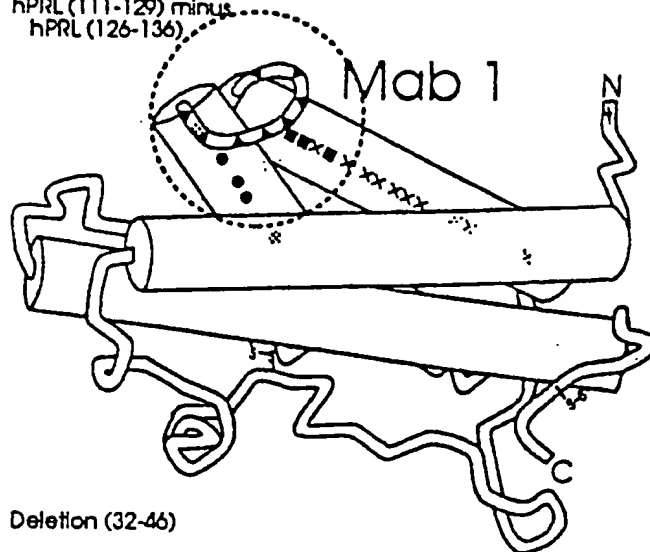
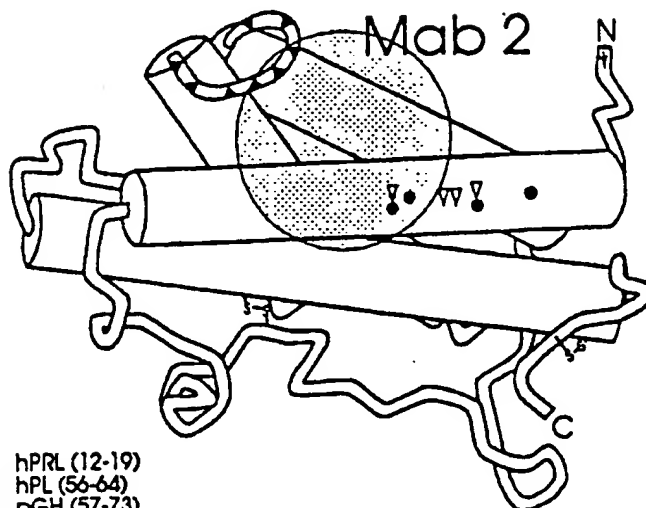


FIG.—5

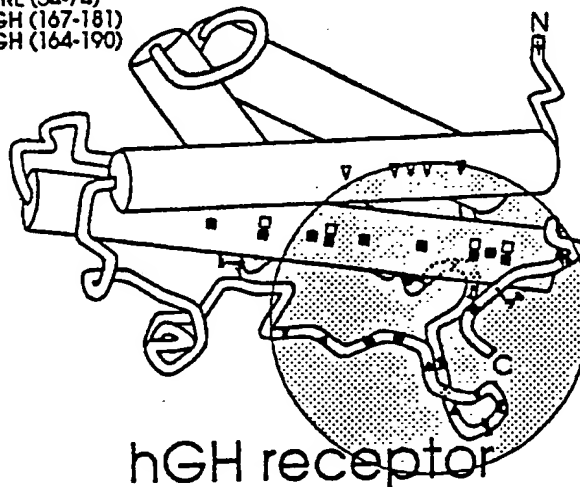
- hPRL (88-95)
- ▲ hPRL (97-104)
- hPL (109-112)
- x hPRL (111-129) minus  
hPRL (126-136)

**FIG.—6A****SUBSTITUTE SHEET**

- pGH (11-33) minus hPRL (22-33)
- ▽ hPRL (12-19) minus hPL (12-25)
- ▲ hPRL (97-104)



- ▽ hPRL (12-19)
- × hPL (56-64)
- ▲ pGH (57-73)
- hPRL (54-74)
- pGH (167-181)
- pGH (164-190)



- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)

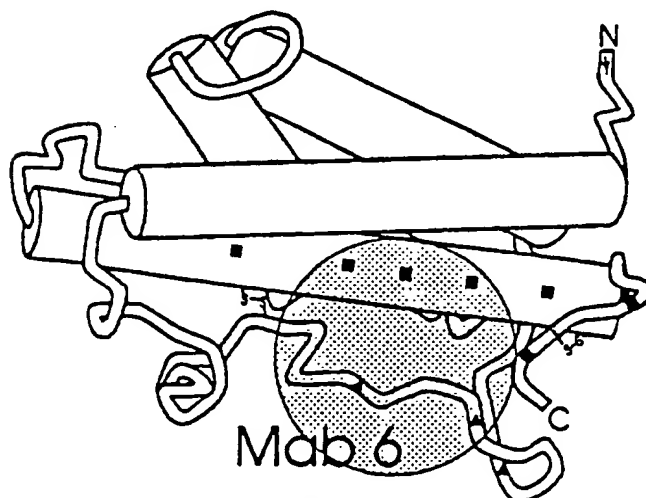
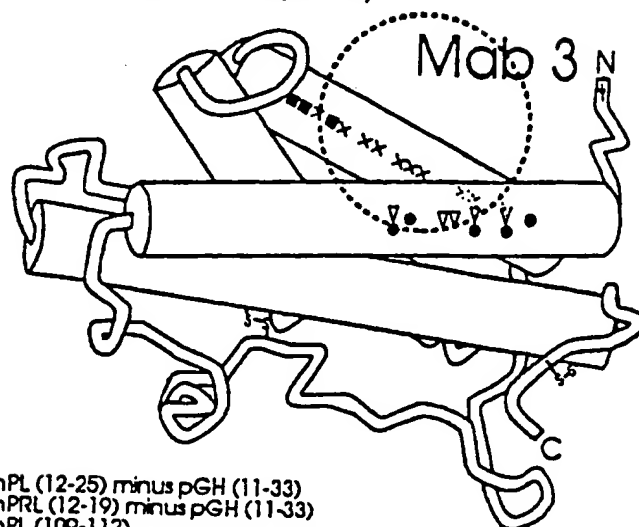


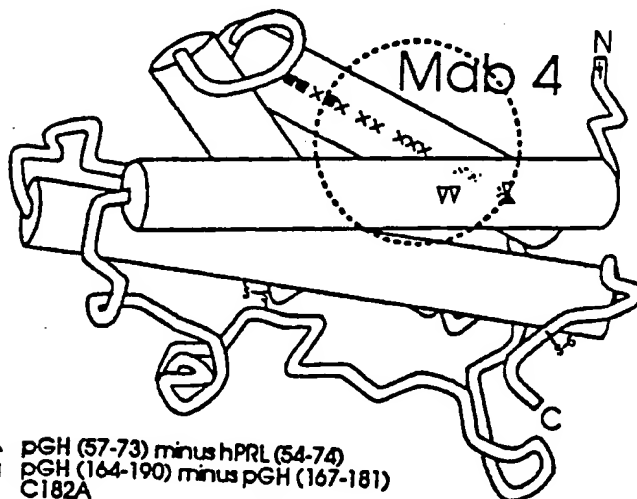
FIG.—6B

SUBSTITUTE SHEET

- pGH (11-33) minus hPRL (22-33)
- ▲ hPL (12-25) minus hPRL (22-33)
- ▽ hPRL (12-79)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)



- ▲ hPL (12-25) minus pGH (11-33)
- ▽ hPRL (12-19) minus pGH (11-33)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)



- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)
- C182A

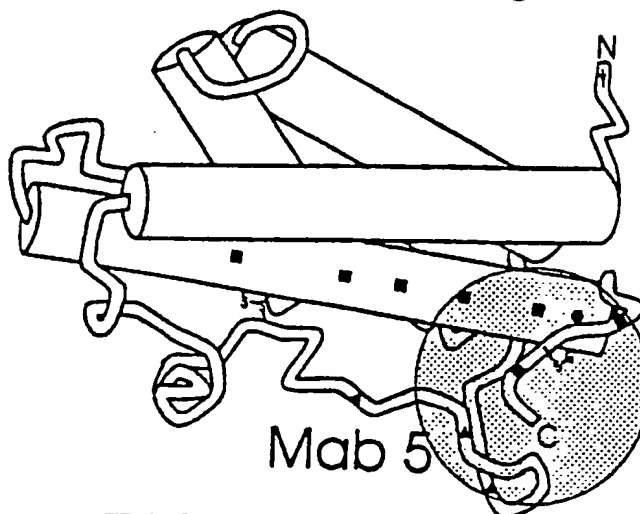


FIG.—6C

SUBSTITUTE SHEET

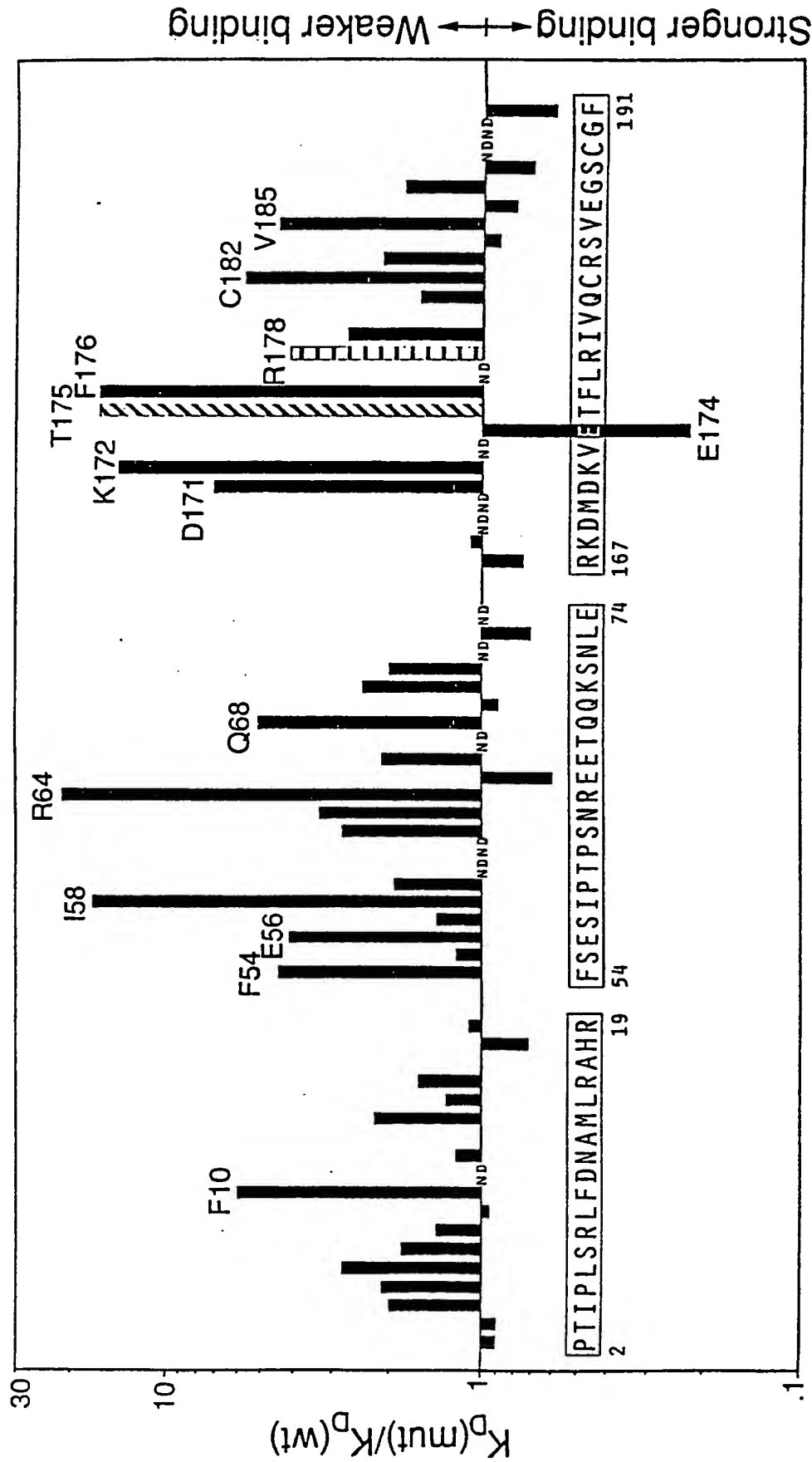


FIG.—7



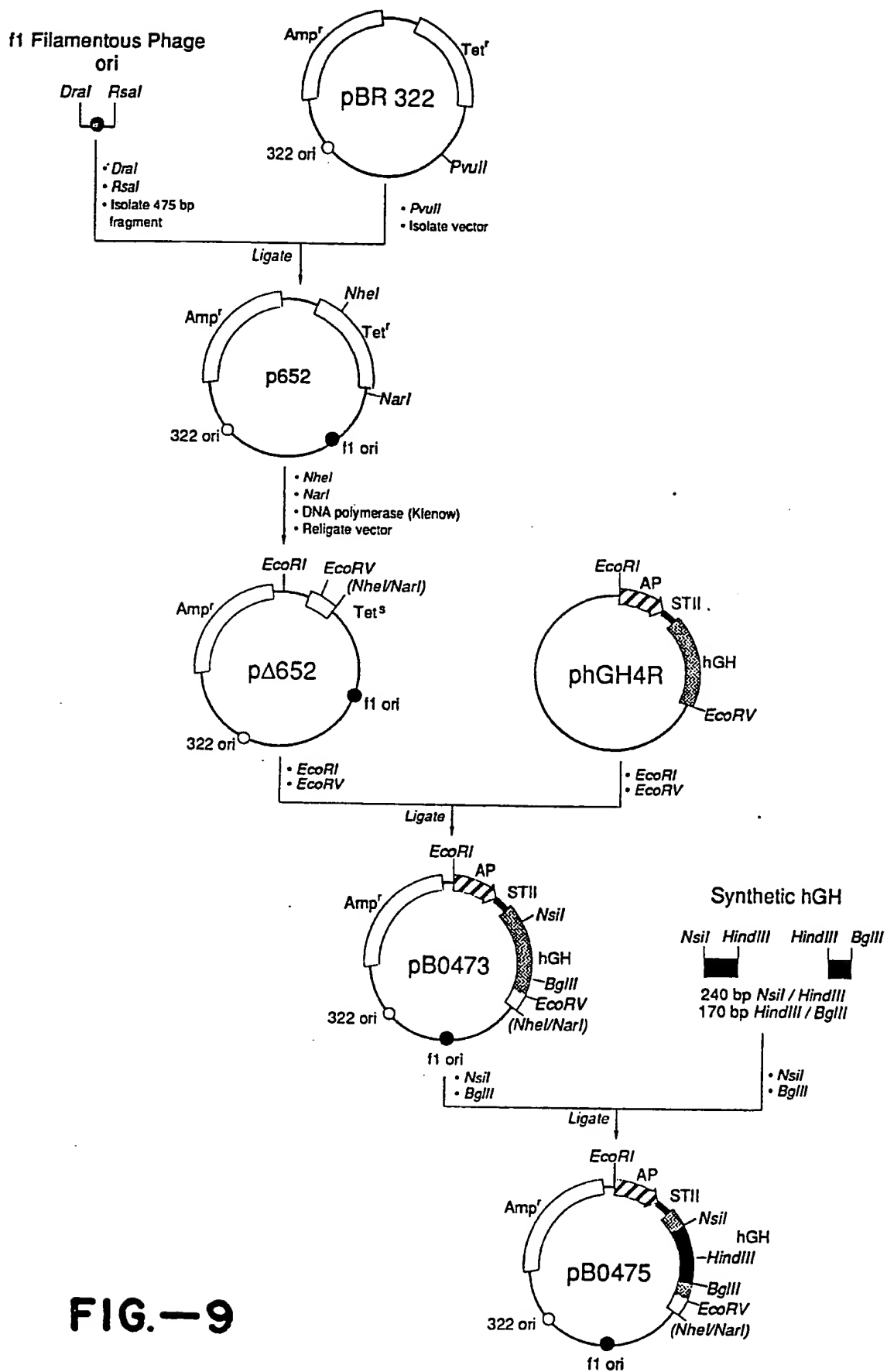
10/55

## hGH Synthetic Gene

-20 Met Lys Lys Asn Ile -Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala  
 ATG AAA AAG AAT ATC GCA TTT CTT CTT CTT GCA TCT ATG TTC GTT TTT TCT ATT GCT ACA AAT GCC TAT GCA  
 -10  
 +1 Phe Pro Thr Ile Pro Leu Ser Arg Leu Phe Asp Asn Ala Met Leu Arg Ala His Arg Leu His Gln Leu Ala  
 TTC CCA ACT ATA CCA CTA AGT CGA CTA TTC GAT AAC GCT ATG CTT CGG GCC CAT CGT CTT CAT CAG CTA GCC  
 30  
 79 Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro  
 TTT GAC ACC TAC CAG GAG TTT GAA GAG GCC TAT ATC CCC AAG GAA CAG AAG TAT TCA TTC CTG CAG AAC CCC  
 40  
 142 Phe Asp Thr Tyr Gln Glu Phe Glu Glu Ala Tyr Ile Pro Lys Glu Gln Lys Tyr Ser Phe Leu Gln Asn Pro  
 TTT GAC ACC TAC CAG GAG TTT GAA GAG GCC TAT ATC CCC AAG GAA CAG AAG TAT TCA TTC CTG CAG AAC CCC  
 50  
 214 Gln Thr Ser Leu Cys Phe Ser Glu Ser Ile Pro Thr Pro Ser Asn Arg Glu Glu Thr Gln Lys Ser Asn  
 CAG ACC TCC CTC TGT TTC TCA GAA TCG ATT CCG ACA CCC TCC AAT CGC GAG GAA ACA CAA CAG AAA TCC AAC  
 60  
 286 Leu Glu Leu Leu Arg Ile Ser Leu Leu Ile Gln Ser Trp Leu Glu Pro Val Gln Phe Leu Arg Ser Val  
 CTA GAG CTC CTC CGC ATA AGC ATA AGC TTT GAG TTT GAG TCG TCG TCG CTC GAG CCC GTG CAG TTC CTG AGG AGT GTC  
 80  
 358 Phe Ala Asn Ser Leu Val Tyr Gly Ala Ser Asp Ser Asn Val Tyr Asp Leu Leu Lys Asp Leu Glu Glu Gly  
 TTC GCC AAC AGC CTG GTC TAC TAC GGC GCC TCT GAT TCG AAC GTG TAC GAC CTG GAC CTG AAG GAC CTA GAG GAA GGG  
 100  
 430 Ile Gln Thr Leu Met Gly Arg Leu Glu Asp Gly Ser Pro Arg Thr Gly Gln Ile Phe Lys Gln Thr Tyr Ser  
 ATC CAA ACG CTG ATG GGG AGG CTG GAA GAT GGC AGC CCG CGG ACT GGG CAG ATC TTC AAG CAG ACC TAC AGC  
 110  
 502 Lys Phe Asp Thr Asn Ser His Asn Asp Asp Ala Leu Leu Lys Asn Tyr Gly Leu Leu Tyr Cys Phe Arg Lys  
 AAG TTC GAC ACA AAC TCA CAC AAC GAT GAC GCA CTA CTC AAG AAC TAC TAC GGG CTG CTC TAC TGC TTC AGG AAG  
 120  
 574 Asp Met Asp Lys Val Glu Thr Phe Leu Arg Ile Val Gln Cys Arg Ser Val Glu Gly Ser Cys Gly Phe AM\*  
 GAC ATG GAC AAG GTC GAG ACA TTC CTG CGC ATC GTG CAG TCG CGC TCT GTG GAG GGC AGC TGT GGC TTC TAG  
 130  
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 850  
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 870  
 880  
 890  
 900  
 910  
 920  
 930  
 940  
 950  
 960  
 970  
 980  
 990  
 1000

FIG.-8

11/55



12/55

[illegible]

**FIG. 10B**

**FIG.-10C**

3

**FIG. 10D**



**FIG. -10F**



**FIG. 10G.**

[illegible]

**FIG. -10H**

20/55

sau3AI fnu4HI  
 mboI(dam-) haeIII  
 dpnI eaeI  
 pvuI cfrI  
 4241 CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTATCAC nlaIII fnu4HI  
 GCGTAGCAAC AGTCTTCATT CAACCGGCGT CACAATAGTG AGTACCAATA CCGTCGTGAC bbvI  
 CATAATTC TC TTAGTGTAT GGCAGCACTG CATAATTC TTACTGTTCAT nlaIII foki  
 AGATGCTTTT sfanI  
 TCTACGAAAA  
 4341 CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA GTGTATCGCG fnu4HI  
 GACACTGACC ACTCATGAGI TGGTTCAGTA AGACTCTTAT CACATACGCC GCTGGCTCAA CGACCGAGTT GCTCTTGTCCC GCGGTCAACA cauII hincII  
 CGGGATATA CCGGCGCACA  
 bsrI rsal  
 hphI scaI  
 bsrI hgiAI  
 draI hgiAI bsp1286 xnnI mboII  
 4441 TAGCAGAACT TTAAGAAGTC TCATCATTTGG AAAACGTTCT TCGGGCGGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT  
 ATCGTCTTGA AATTTCACG AGTAGTAACC TTTTGCAAGA AGCCCCGCTT TTGAGAGTTC CTAGAATGG CACAACCTCTA GGTCAGCTA CATGGGTGA  
 msel  
 hgiAI mboII(dam-) bsrI  
 sau3AI  
 bsp1286 sau3AI  
 apalI mboI(dam-) mboI(dam-) dpnI  
 4541 CGTGACCCCA CTGATCTTC AGCATCTTTT ACTTTCACCA hphI hphI fnu4HI  
 GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT CGCAAGAGCC CACTCGTTTT TGTCCTTCGG TTTTACGGCG TTTTTCCT TATCCCGCT  
 sfanI  
 mboII  
 earI  
 4641 CACGGAATG TTGAATACTC ATACTCTTCC TTTTTCATA sspl  
 GTGCTTTAC AACTATGAG TATGAGAAGG AAAAAGTTAT AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCT ATGTATAAC TACATATTTG AATGATTTA  
 bsmalI  
 nlaIII  
 bspHI

FIG.-101

2/55

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4741 GGAATAATAA CAAATAGGGG TCCGCGGCAC ATTCCCGCA AAGTGCCAC CTGACGTCTA AGAACCAAT ATTATCATGA CATTAAACCTA TAAAAATAGG
CTTTTATTT GTTATATCCC AAGCGCGTG TAAAGGGCT TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATGGAT ATTTTATCC

          hinPI          nlaIII          mspI
          hhai          bspHI
          thai          acyI ddeI
          fnuDII          aatII
          nlaIV bstUI[M.hhaI-]
          sau96I[M.haeIII-]
          haeIII
          asuI
          ecoO109I
          mnlI          mboII
          4841 CATTATCACA GGCCTTTTCG TCTTCRA
          GCATAGTGCT CCGGAAGC AGAAGTT

>length: 4867

aa+II(GAGGTC):
accI(GTACAC):
accIII(TCCGGA):
acyI(GRCGYC):
ahaII(GRCGYC):
ahaIII(TTTAAA):
alul(AGCT):
alwi(GGATC):
alwNI(CAGNNCTG):
apaI(GGGCC):
apaLI(GTGAC):
aseI(ATTAAAT):
asuI(GGACC):
asuII(TTCGAA):
avaI(CYCGRG):
avaII(GGACC):
avaIII(ATGCAT):
bali(TGGCCA):
bamHI(GGATCC):
bani(GGYRCC):
banII(GRCGYC):
bbvI(GCAGC):
bclI(dam-)(TGATCA):

          nlaIII          mspI
          bspHI
          acyI ddeI
          aatII
          CTGACGTCTA AGAACCAAT ATTATCATGA CATTAAACCTA TAAAAATAGG
          GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATGGAT ATTTTATCC
          AAGTGCCAC CTGACGTCTA AGAACCAAT ATTATCATGA CATTAAACCTA TAAAAATAGG
          TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATGGAT ATTTTATCC
          TAAAGGGCT TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATGGAT ATTTTATCC
          TCTTCRA
          CCGGAAGC AGAAGTT
          4793
          477[M.taqI-] 761 2753
          1701 2108 2568
          767 4411 4793
          767[M.hhaI-] 4411[M.hpaII-] 4793
          3739 3758 4450
          72 203 271 522 678 692 1019 1032 1040 2036 2093 2166 2423 2623 2642 2923 3149
          3239 3285 3542 4063 4163 4226
          816 817 1704 2105 2106 2571 2572 3549 3623 3635 3720 3733 4197 4500 4518
          728 3393
          504
          2798 3296 4542
          4046
          504 505 802 1182 1297 1476 1518 1797 1986 2328 3917 3996 4013 4235 4851
          778
          716[M.taqI-] 1462
          802 1476[dcM-] 1518 1797 4013 4235
          453
          1481[dcM-]
          816 2105[M.mspI-] 2571[M.mspI-]
          767 1086 1129 1326 2374 3823
          504[M.haeIII-] 677[M.aluI-] 719 2408
          204 207 697 849 940 1017 1033 1236 1443 1467 1596 1599 1722 2516 2621 2718 2887
          2905 3324 3389 3392 3598 3926 4115 4292
          138

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FIG.-10J

22/55

b9LI(GCCNNNNNGGC):  
 b9LII(AGATCT):  
 b9MI(GAATGC):  
 b9MI(GTCTC):  
 b9P1285(ODGCHC):  
 b9PHI(TCATA):  
 b9PMI(ACTGCG):  
 b9PMI(TCCGGA):  
 b9PI(ACTGG):  
 b9PI(TTCGAR):  
 b9PMI(CCWGG):  
 b9PMI(GCGG):  
 b9XI(CCAANNNTTGG):  
 b9YI(AGATCY):  
 b9J36I(CCTNAGG):  
 b9MI(CCSGG):  
 b9PI(YGGCCR):  
 b9AI(ATCGAT):  
 b9AI(CTNAG):  
 b9AI(GATC):  
 b9AI(TTTAA):  
 b9AI(CACNNNGTG):  
 b9AI(YGGCCR):  
 b9AI(CGGCCG):  
 b9AI(CTCTTC):  
 b9BI(CCTNAGG):  
 b9NI(CCTNNNNAGG):  
 b9O109I(RGGNCCY):  
 b9RI(GAATTC):  
 b9RI(CCWGG):  
 b9RV(GATATC):  
 b9HI(GCMGC):  
 b9DII(CGCG):  
 b9KI(GGATG):  
 b9PI(TGCGCA):  
 b9I(WGGCCW):  
 b9I(RGGCGY):  
 b9II(GGCC):  
 3989[M.haeIII-]  
 867  
 182 455 1390  
 295 977 2631 3942 4707  
 504[M.haeIII-] 719 1502 2408 2798 3296 4457 4542  
 3702 4710 4815  
 792  
 1701 2108 2568  
 706 860 1220 1547 1818 1842 2250 2729 2757 3385 3398 3515 3921 4039 4082 4346  
 4521  
 778  
 541 757 1140 1479 3009 3130 3143  
 211[M.hhaI-] 647 855 1271 1281 1426 1452 1574 1671 2043 2144 2520[M.hhaI-] 2540[M.hhaI-]  
 2564[M.hhaI-] 2582[M.hhaI-] 2584[M.hhaI-] 2687[M.hhaI-] 3028 3609[M.hhaI-] 3939  
 4432[M.hhaI-] 4764[M.hhaI-]  
 750  
 816 867 1704 2105 2571 3623 3634 3720 3732 4500 4517  
 733  
 1180 1295 1521 1849 2627 2662 3361 4057 4408  
 290 1481 4263  
 625  
 57 473 619 734 1618 1780 2792 3257 3666 3832 4372 4798  
 139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197  
 4243 4501 4518 4554  
 3739 3758 4450  
 2332  
 290 1481 4263  
 290  
 551 2860 4664  
 733  
 793  
 801 1475[dcn-] 1517 4850  
 1  
 541 757 1140 1479 3009 3130 3143  
 1195  
 204 207 697 849 940 1002 1017 1033 1236 1245 1324 1443 1446 1453 1467 1596 1599  
 1722 1803 2516 2538 2552 2621 2718 2771 2887 2905 2908 3026 3181 3324 3389 3392  
 3598 3926 4115 4265 4292 4387 4616  
 211 647 855 1271 1281 1426 1452 1574 1671 2043 2144 2520 2540 2564 2582 2584  
 2687 3028 3609 3939 4432 4764  
 238 703 1122 1143 1718 1807 1885 2046 2657 3855 4036 4323  
 987 1393 1491 4095  
 555 1481 2995 3006 3458  
 153 767 1242 1681 1764 2484 2492 2856 3226  
 291 505 556 1183 1298 1482 1986 2186 2328 2996 3007 3025 3459 3917 3997 4264  
 4851

FIG.—10K

23/55

hgaI(GAGCC):  
 hgiAI(GGCGWC):  
 hgiCI(GGRC):  
 hgiJII(GRGCCY):  
 hhaI(GCGC):  
 hnpI(GCGC):  
 hncII(GTYRAC):  
 hindII(GTYRAC):  
 71 691  
 hndIII(GAGCTT):  
 hnfII(GATC):  
 hpaII(CCGG):  
 hphI(GGTGA):  
 hhoII(GAAG):  
 hpiI(dam-)(GATC):  
 hmlI(CCTC):  
 mfeI(TTA):  
 mspI(CCGG):  
 mstI(TGCGCA):  
 mstII(CCTNAGG):  
 naeI(GCCGGC):  
 narI(GCGGCC):  
 nciI(CCSGG):  
 ndeI(CAATG):  
 nheI(GCTAGC):  
 nlaIII(CATG):  
 nlaIV(GGNCC):  
 nrui(TCGCGA):  
 nsiI(ATGCAT):  
 nspCIX(RCATGY):  
 paer7I(CTCGAG):  
 pflMI(CCANNNNTGG):  
 pleI(GAGTC):  
 ppuMI(RGGWCCY):  
 pstI(CTGCAG):  
 917 1277 1427 2041 2565 2688 3084 3662 4412  
 677[M.aluI-] 1502 2798 3296 4457 4542  
 767 1086 1129 1326 2374 3823  
 504 677 719 2408  
 112 154 210 768 988 1111 1243 1394 1456 1492 1682 1765 2485 2493 2519 2541 2550  
 2563 2583 2686 2716 2857 2890 3160 3227 3327 3501 3610 4003 4096 4433 4765  
 112 154 210 768 988 1111 1243 1394 1456 1492 1682 1765 2485 2493 2519 2541 2550  
 2563 2583 2686 2716 2857 2890 3160 3227 3327 3501 3610 4003 4096 4433 4765  
 477[M.taqI-] 4414  
 477 4414  
 623[M.taqI-] 628[M.taqI-] 776[M.taqI-] 1341[M.hphI-] 1562[M.hphI-] 2068 2264  
 2286 2882 2957 3353 3870  
 1171 1180 1295 1321 1522 1702 1849 2109 2439 2569 2628 2662 3189 3336 3362 3552  
 3956 3990 4057 4167 4409  
 380 1136 1344 1565 2346 2592 2601 3726 3953 4349 4575 4590  
 409 514 551 744 842 870[dam-] 1638 2465 2861 3632[dam-] 3723[dam-] 4478 4556[dam-]  
 4665 4861  
 139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197  
 4243 4501 4518 4554  
 148 163 241 372 378 554 606 610 639 650 682 736 771 809 835 1013 1125 1185 1265  
 1303 1330 1516 1830 1888 1944 2372 2579 2609 2871 3097 3154 3421 3821 3902 4032  
 4238 4849  
 69 257 324 1044 1066 1757 1979 2011 2125 2136 2148 2159 2176 2274 2545 2763  
 3688 3740 3745 3759 3812 4047 4086 4451 4823  
 1171 1180 1295 1321 1522 1702 1849 2109[M.bamHI-] 2439 2569[M.bamHI-] 2628 2662  
 3189 3336 3362 3552 3956 3990 4057 4167 4409  
 987 1393 1491 4095  
 733  
 1320 2438  
 767  
 1180 1295 1521 1849 2627 2662 3361 4057 4408  
 2804  
 523[M.aluI-] 1239  
 40 964 1288 1495 1629 1854 1918 1983 2618 2723 2983 3703 4194 4204 4282 4318  
 4711 4816  
 504 767 816 1086 1129 1291 1326 1361 1475 1518 1797 2105 2374 2395 2407 2571  
 3012 3051 3823 3917 3958 4169 4759  
 646  
 453  
 1853 2617 2982  
 716  
 14 1352 1401  
 2264 2286 2882 3353 3870  
 801 1475 1517  
 590 4116[M.HI-]

FIG.-10L

24/55

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PstI(CGATCG):
PvuII(CAGCTG):
KpnI(GTAC):
SacI(GAGCTC):
SacII(CCGCGG):
SalI(GTCGAC):
SmaI(GATC):
Sau96I(GGATCC):
4242
270 1018[M.HI-]
159 342 787 1174 2789 4354
677
854
477
139 817 868 1498 1705 2106 2572 3549 3624 3635 3643 3721 3733 3838 4179 4197
4243 4501 4518 4554
504[M.haeIII-] 505[M.haeIII-] 802 1182[M.haeIII-] 1297[M.haeIII-] 1476[dcM-]
1518 1797 1986[M.haeIII-] 2328[M.haeIII-] 3917[M.haeIII-] 3996[M.haeIII-] 4013
4235 4851[M.haeIII-]
4353
1180 1295 1521 1849 2627 2662 3361 4057 4408
541 757 1140 1479 3009 3130 3143
175 237 416 990 1144 1214 1458 1710 1719 1806 1884 1947 2658 2774 2829 2850
3070 4122 4332 4562
217
338
2127 4677
677
535
567 1406
478 486 626[M.claI-] 717 779 894 975 1305 2370 3082 4526
211 647 855 1271 1281 1426 1452 1574 1671 2043 2144 2520 2540 2564 2582 2584
2687 3028 3609 3939 4432 4764
968 2726
368
716
816 867 1704 2105 2571 3623 3634 3720 3732 4500 4517
290
623 2068 4470
not found:
PstI(CTTAAAG), asp718(GGTACC), avrII(CCTAGG), bssHII(GCGGCG), bsteII(GGTNACC), espi(GCTNAGC), hpaI(GTTAAC),
kpnI(GGTACC), mluI(ACGCGT), ncoI(CCATGG), notI(GCGGCGG), rsrII(CGGWCCG), sfiI(GGCCNNNNNGGCC), smaI(CCCGGG),
sphi(GCATGC), xmaI(CCCGGG)

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FIG.-10M

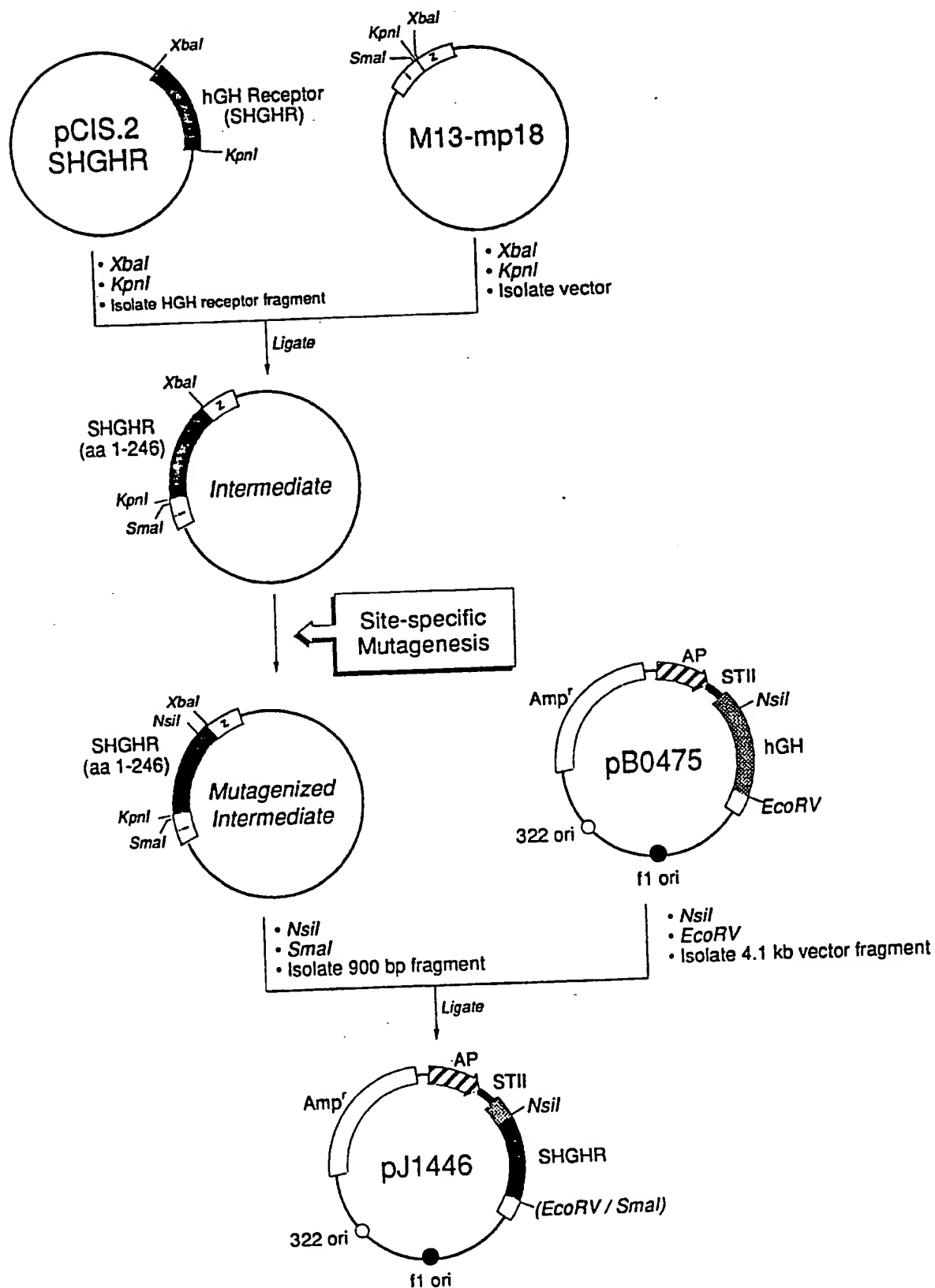


FIG.—II

SUBSTITUTE SHEET



26/55

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1 GAATTCAACT TCTCCATACT TTGGATAAGG AATPACAGAC ATGAAAATC TCATTGCTGA ddeI
CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGCTG TACTTTTAG AGTAACGACT CAACATAAA TTCGAACCT CTAATAGCAG TGACGTTACG
          aluI
          hindIII
          mseI
          bsmI
101 TTGCGCAATAT GCGGCAAAAT GACCAACAGC GGTGATTGA TCAGGTAGAG GGGGCGGTGT ACAGGATAAA GCCGATGCC AGCATTCCTG ACGACCGATAC
TAGCGTTATA CCGGTTTTA CTGGTGTGCG CCAACTAACT AGTCCATCTC CCCCAGCACA TGCTCCATTT CGGCTACGG TCGTAAGGAC TGCTGCTATG
          sau3AI
          mboI[dam-]
          hinPI
          hhaI
          bclI[dam-]
          mnlI
          haeII
          rsaI
          fnu4HI
          bbvI
          fnuDII
          fnu4HI
          bstUI[M.hhaI-]
          bbvI
          hinPI
          aluI
          hhaI
          snaBI
          foki
          sfanI
          mnlI
          mseI
          pvuII
          aluI
          bsmI
          cfrI
          bsmalI
201 GGAGCTGCTG CCGGATTACG TAAGAAGTT ATTGAAGCAT CCTCGTCAGT AAAAGCTTAA TCTTTTCAAC AGCTGTCTATA AAGTTGTCTAC GGCCGAGACT
CCTCGACGAC CGCTAATGC ATTCTTCAA TAACCTCGTA GGAGCAGTCA TTTTTCATTT AGAAAAGTTG TCGACAGTAT TTCAACAGTG CCGGCTCTGA
          thai
          fnu4HI
          bbvI
          fnuDII
          fnu4HI
          bstUI[M.hhaI-]
          bbvI
          hinPI
          aluI
          hhaI
          snaBI
          foki
          sfanI
          mnlI
          mseI
          pvuII
          aluI
          bsmI
          cfrI
          bsmalI
301 TATAGTCGCT TTGTTTTTAT TTTTAAATGT ATTTGTAAGT AGTACGCAAG TTCACGTAAA AAGGGTATCT AGAGGTTGAG GTGATTTT
ATATCAGCGA AACAAAATA AAAAATTACA TAACATTTGA TCATCGGTTT AAGTGCATTT TTCCCATAGA TCTCCAATC CACTAAAA
          mseI
          speI
          rsaI
          xbaI
          mnlI
          mnlI
          bphI
          ATG AAA
          TAC TTT
          Met Lys
          haeIII
          haeI
          mnlI
          nsII
          avallI
          TAT GCA TTT TCT GGA AGT GAG GCC
          TAT GCA TTT TCT GGA AGT GAG GCC
          ATA CGT AAA AGA CCT TCA CTC CGG
          Tyr Ala Phe Ser Gly Ser Glu Ala
          haeIII
          stuI[dcM-]
          haeI
          scrFI[dcM-]
          ecorII
          bstNI
          mseI
          bstNI
          CTA AAG ACA AAT TCT TCT AAG GAG CCT
          CCA GGC CTA AAG ACA AAT TCT TCT AAG GAG CCT
          TTA GGT CCG GAT TTC TGT TTA AGA TTA CTC GGA
          Val Asn Pro Gly Leu Lys Thr Asn Ser Ser Lys Glu Pro
          pleI
          hinFI
          scrFI[dcM-]
          hgiAI
          ecorII
          bstNI
          bspI286
          ddeI
          aluI
          fnu4HI
          bbvI
          ddeI
          CTT AGC GCA CCC TGG AGT CTG CAA AGT GTT AAT CCA GGC CTA AAG ACA AAT TCT TCT AAG GAG CCT
          TAG GAA TCG TCT CGT GGG ACC TCA GAC GTT TCA CAA TTA GGT CCG GAT TTC TGT TTA AGA TTA CTC GGA
          Ile Leu Ser Arg Ala Pro Trp Ser Leu Gln Ser Val Asn Pro Gly Leu Lys Thr Asn Ser Ser Lys Glu Pro
          7 Thr Ala Ala Ile Leu Ser Arg Ala Pro Trp Ser Leu Gln Ser Val Asn Pro Gly Leu Lys Thr Asn Ser Ser Lys Glu Pro

```

FIG.-12A

draIII hphi hphiI ddei bsmal nlaIII bsri mnli nlaIII rsal styI  
 557 AAT TTC ACC AAG TGC CGT TCA CCT GAG CGA GAG ACT TTT TCA TGC CAC TGG ACA GAT GAG GGT CAT GGT ACA AAG AAC  
 TTT AAG TGG TTC ACG GCA AGT GGA CTC GCT CTC TGA AAA AGT ACG GTG ACC TGT CTA CTC CAA GTA CCA TGT TTC TTG  
 34 Lys Phe Thr Lys Cys Arg Ser Pro Glu Arg Glu Thr Phe Ser Cys His Trp Thr Asp Glu Val His His Gln Thr Lys Asn  
 sau96I  
 nlaIV  
 avaiI  
 asuI  
 ppuMI aluI  
 638 CTA GGA CCC ATA GAG CTG TTC TAT ACC AGA AGG AAC ACT CAA GAA TGG ACT CAA GAA TGG AAA TGC CCT GAT TAT GGT  
 GAT CCT GGG TAT GTC GAC AAG ATA TGG TCT TCC TGA GTT CTT ACC TGA GTT CTT ACC TTT CTT ACG GGA CTA ATA CAA  
 61 Leu Gly Pro Ile Gln Leu Phe Tyr Thr Arg Arg Asn Thr Gln Glu Trp Thr Thr Gln Glu Trp Lys Glu Cys Pro Asp Tyr Val  
 aluI  
 pvuII  
 719 TCI GCT GGG GAA AAC AGC TGT TAC TTT AAT TCA TCG TTT ACC TCC ATC TGG ATA CCT TAT TGT ATC AAG CTA ACT AGC AAT  
 AGA CGA CCC CTT TTG TCG ACA ATG AAA TTA AGT AGC AAA TGG AGG TAG ACC TAT GGA ATA ACA TAG TTC GAT TGA TCG TTA  
 88 Ser Ala Gly Glu Asn Ser Cys Tyr Phe Asn Ser Ser Phe Thr Ser Ile Trp Ile Pro Tyr Cys Ile Lys Leu Thr Ser Asn  
 these first 9 bases differ from 262 and 265  
 msei  
 mnli  
 sau3AI  
 mboI[dam-]  
 dpnI  
 aluI  
 xhoII  
 bstYI  
 800 GGT GGT ACA GTG GAT GAA AAG TGT TTC TCT GAT GAA ATA GTG CAA CCA GAT CCA CCC ATT GCC CTC AAC TGG TGG ACT TTA  
 CCA CCA TGT CAC CTA CTT TTC ACA AAG AGA CAA CTA CTT TAT CAC GTT GGT CTA GGT GGG TAA CGG GAG TTG ACC IGA AAT  
 115 Gly Thr Val Asp Glu Lys Cys Phe Ser Val Asp Glu Ile Val Gln Pro Asp Pro Pro Ile Ala Leu Asn Trp Thr Thr  
 rsal foki mnli bsri  
 881 CTG AAC GTC AGT TTA ACT GGG ATT CAT GCA GAT ATC CAA GTG AGA TGG GAA GCA CCA CGC AAT GCA GAT ATT CAG AAA GGA  
 GAC TTG CAG TCA AAT TGA CCC TAA GTA CGT CTA TAG GTT CAC TCT ACC CTT CGT CGT GGT TTA CGT CTA TAA GTC TTT CCT  
 142 Leu Asn Val Ser Leu Thr Gly Ile His Ala Asp Ile Gln Val Arg Trp Glu Ala Pro Arg Asn Ala Asp Ile Gln Lys Gly  
 msei  
 sau96I  
 nlaIV  
 avaiI  
 asuI  
 962 TGG ATG GTT CTG GAG TAT GAA CTT CAA TAC AAA GAA GTA AAT GAA ACT AAA TGG AAA ATG ATG GAC CCT ATA TTG ACA ACA  
 ACC TAC CAA GAC CTC ATA CTT GAA GTT ATG TTT CTT CAT TTA CTT TGA TTT ACC TTT TAC TAC CTG GGA TAT AAC TGT TGT  
 169 Trp Met Val Leu Glu Tyr Glu Leu Gln Tyr Lys Glu Val Asn Glu Thr Lys Trp Lys Met Met Asp Pro Ile Leu Thr Thr  
 foki  
 sau96I  
 nlaIV  
 avaiI  
 asuI

sau3AI  
 mboI[dam-]  
 dpnI  
 alwI  
 xhoII  
 bstYI  
 bsrI  
 rsal  
 1043 TCa GTT CCa GTG TAc TCa TTG AAA GTG GAT AAG GAA TAT GAA GTG CGT GTG AGA TCC AAA CAA CGA AAC TCT GGa AAT TAT  
 AGT CAA GGI CAC AIG AGT AAC TTT CAC CTA TTC CTT ATA CTT CAC GCA CAC TCT AGG TTT GTT GCT TTG AGa CCT TA ATA  
 196 Ser Val Pro Val Tyr Ser Leu Lys Val Asp Lys Glu Tyr Val Arg Val Arg Ser Lys Gln Arg Asn Ser Gly Asn Tyr  
 haeIII  
 xmaIII  
 eagI  
 eaeI  
 cfrI  
 notI  
 fnu4HI  
 CGC CTC AAG TCA CTC CAC GAG ATA CAT TGT GAA GGA GTC TAC TCG GTT AAA TGT ACA CTT CTT CTA AAG ATG ATC GC  
 223 Gly Glu Phe Ser Glu Val Leu Tyr Val Thr Leu Pro Gln Met Ser Gln Phe Thr Cys Glu Glu Asp Phe Tyr AM\*  
 mseI  
 hpaI  
 hindII  
 hincII  
 thai  
 fnuDII  
 bstUI  
 fnu4HI  
 1201 GCCGCGTTAA CTGTTTATT GCAGCTTATA ATGTTTACAA ATAAGCAAT AGCATCACAA ATTTACAAA TAAAGCATTT TTTTCACTGC ATTCTAGTTG  
 CGGCGCAATT GAACAAATAA CGTCGAATAT TACCAATGTT TATTTCGTTA TCGTAGTGT TAAAGTGT TTTCGTAATAA AAAAGTGACG TAAGATCAAC  
 sau3AI  
 mboI[dam-]  
 dpnI  
 alwI  
 xhoII  
 nlaIV  
 bstYI  
 bamHI  
 nlaIII alwI  
 1301 TGGTTTGTC AACTCATCA ATGTATCTTA TCATGTCCTG ATCCCATCGT CCATTCCGAC AGCATCGCA GTCACATAGG GTCACATAGG CGTCTGCTA GCGCCGCCCT  
 ACCCAACAGG TTGAGTAGT TACATAGAAT AGTACAGACC TAGGTAGCA GGTAAGGCTG TCGTAGCGGT CAGTGATACC GCACGACGAT CCGCGCGGGA  
 fnu4HI  
 nheI  
 hinPI  
 fnu4HI  
 bbvI  
 haeII  
 bsmI

[illegible]

[illegible]

W-21-G-FIG



scrFI  
 nciI  
 mspI  
 hpaII  
 sfaNI  
 foki cauII  
 2801 AAGCGGATGC CGGGGCGAGA CAAGCCCGTC AGGCGGCGTC AGCGGGTGTG GCGGGGTGTC GGGCGCAGC CATGACCCAG TCACGTAGCG ATAGCGGAGT  
 TTCGCTACG GCCCTCGTCT GTTCGGGCGAG TCCCGGCGAG TCGCCACAA TCGCCACAG CGGCCACAG CCGCGCGTCG GTACTGGGTC AGTCATCGC TATCGCCTCA

bsrI  
 accI  
 2901 GTATACTGGC TTAACATATGC GGCATCAGAG CAGATTGTAC TGAGAGTGCA CCATATGCGG TGTGAAATAC CGCACAGATG CETAAGGAGA AAATACCGCA  
 CATAGACCG AATTGATACG CCGTAGTCTC GTCTAACATG ACTCTACGT GGTATACGCC ACACCTTATG CCGTGTCTAC GCATTCCTCT TTTATGGCGT

mboII  
 eari  
 hinPI  
 hhaI  
 haeII  
 3001 TCAGCGGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG CTCGGTCTGT CGGCTGCGGC GAGCGGATC AGCTCACTCA AAGGCGGTAA TACGGTTATC  
 AGTCCGGGAG AAGGCGGAGG AGCGAGTGAC TGAGCGACGC GAGCCAGCAA GCCGACGCCG CTCGCCATAG TCGAGTGAGT TTCCGCCATT ATGCCAATAG

hinFI  
 3101 CACAGATCA GGGGATAACG CAGGAAGAA CATGTGAGCA AAGGCCAGC AAGCCGTAA GAACCGGCT TCGTGGCGGT TTTCCATAGG nlaIV  
 GTGTCTAGT CCCCTATTGC GTCCCTTCTT GTACACTCGT TTTCGGGTC TTTTCGGTC AATCCGATTT TTCCGGCGCA ACACCGCAA AAAGGTATCC

3201 CTCGGCCCC CTGACGAGCA TCACAAAAT CGACGCTCAA GTCAGAGTG GCGAAACCG ACAGGACTAT AAGATACCA GCGGTTTCCC CCTGGAAGCT  
 GAGGCGGGG GACTGCTCGT AGTGTTTGA GTGCGGAGT CAGTCTCCAC CGCTTTGGC TGTCTGATA TTTCTATGT CCGCAAAGG GGACCTTCA

3301 CCCTCGTGG CTCTCTGTGT CCGACCTGTC CGCTTACCGG ATACCTGTCC GCCCTTCTCC CTTCCGGGAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG  
 GGGAGCACGC GAGAGGACAA GGCTGGGACG CGGATGGCC TATGGACAGG CGGAAGAGG GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGGGACATC

FIG.-12G

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**FIG.-12H**



[illegible]

**FIG. -121**

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35/33

## SUBSTITUTE SHEET

```

          bsri
          sau3AI
          mboI{dam-}
          dpnI
          xhoII
          bstYI
          alwI
          hgiAI
          bsp1286
          xnnI
          mboII
          4601 AAAAGTGTCTC ATCAATTGGAA AACGTTCTTC GGGGGGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTGATGT AACCCACTCG TGCACCCAAAC
          TTTTCACGAG TAGTAACCTT TTGCAGAAG CCCCCTTTT GAGAGTTTCT AGAATGGCGA CAACCTCTAGG TCAAGCTACA TTGGGTGAGC ACGTGGGTTG
          mboII{dam-}
          sau3AI
          mboI{dam-}
          dpnI
          sfaNI
          hphI
          hphI
          4701 TGATCTTCAG CAICTTTTAC TTTCACGAGC GTTTCCTGGT GAGCAAAAAC AGGAAGGCAA AATGCCCGCAA AAAAGGGGAAT AAGGGCGGACA CGGAATATGTT
          ACTAGAACTC GT:GAAAATG AAGTGTGTCG CAAGAGACCCA CTCGTTTTTG TCCTTCCGTT TTACGGCGGT TTTTCCCTTA TTCCCGCTGT GCCTTACAA
          mboII
          earI
          sspl
          4801 GAATACTCAT ACTCTTCCTT TTTCATATTT ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTGAA TGTATTTAGA AAAATAAACA
          CTTATGAGTA TGAAGAAGAA AAGTTATAA TAACCTTCCTA AATAGTCCCA ATAACAGAGT ACTCGCCTAT GTATAAACTT ACATAAATCT TTTTATTGT
          hinPI
          hhai
          fnuDII
          nlaIV bstUI{M.hhai-}
          4901 AATAGGGGT CCGCGCACAT TTCCCGGAAA AGTGCCACCT GACGCTTAAG AAACCATTAAT TATCATGACA TTAACCTATA AAAATAGGCG TATCAGCAGG
          TTATCCCAAA GCGCGGTGTA AAGGGCTTT TCACGGTGGA CTGCAGATTC TTGGTAATA ATAGTACTGT AATGGATAT TTTTATCCGC ATAGTGCTC
          mboII
          5001 CCCTTTCGTC TTCA
          GGGAAAGCAG AAGTT
          length: 5015
          aatII(GACGTC): 4941
          accI(GTNKAC): 2901
          accII(TCCGA): 1849 2256 2716
          acyI(GRCGYC): 4559 4941
          ahaII(GRCGYC): 4559[M.hpaiI-] 4941
          ahaII(TTTAAA): 3887 3906 4598
          aluI(AAGT): 72 203 271 481 651 734 786 1223 2184 2241 2314 2571 2771 2790 3071 3297 3387
          3433 3690 4211 4311 4374

```

FIG.-12J

36/55

aaiI(GGATC): 851 1095 1339 1340 1852 2253 2254 2719 2720 3697 3771 3783 3868 3881 4345 4648  
 4666  
 aaiII(CAGNNACGTG): 3541  
 apaII(GIGCAC): 2946 3444 4690  
 aseI(ATTAT): 4194  
 asuI(GGNCC): 641 1024 1445 1624 1666 1945 2134 2476 4065 4144 4161 4383 4999  
 1610  
 avaiI(CYCGRG): 641 1024 1624[dcn-] 1666 1945 4161 4383  
 453  
 avaiII(ATGCAT): 637  
 avriI(CCTAGG): 1629[dcn-]  
 bali(TGGCCA): 1339 2253[M.mspI-] 2719[M.mspI-]  
 1474 2522 3971  
 2556  
 204 207 479 1221 1384 1591 1615 1744 1747 1870 2664 2769 2866 3035 3472  
 3537 3540 3746 4074 4263 4440  
 138  
 bclI(dam-)(TGATCA): 4137[M.haeIII-]  
 bglI(GCCNNNNGGC): 182 701 1289 1538  
 bsmI(GAATGC): 295 587 2779 4090 4855  
 bemaI(GTCTC): 495 1139 1650 2556 2946 3444 4605 4690  
 bspI286(GDGCHC): 3850 4858 4963  
 bspHI(TCATGA): 1849 2256 2716  
 bspvII(TCCGGA): 603 870 896 1049 1368 1695 1966 1990 2398 2877 2905 3533 3546 3663 4069 4187  
 bsrI(ACGG): 4230 4494 4669  
 bstNI(CCWGG): 501 524 1627 3157 3278 3291  
 bstUI(CGCG): 211[M.hhaI-] 1203 1419 1429 1574 1600 1722 1819 2191 2292 2688[M.hhaI-] 2688[M.hhaI-]  
 2712[M.hhaI-] 2730[M.hhaI-] 2732[M.hhaI-] 2835[M.hhaI-] 3176 3757[M.hhaI-] 4087  
 4580[M.hhaI-] 4912[M.hhaI-]  
 bstYI(RGATCY): 850 1094 1339 1852 2253 2719 3771 3782 3868 3880 4648 4665  
 cauII(CCSGG): 1443 1669 1997 2775 2810 3509 4205 4556  
 cfrI(YGGCCR): 290 1199 1629 4411  
 ddei(CTNAG): 57 488 546 579 1158 1766 1928 2940 3405 3814 3980 4520 4946  
 dpnI(GATC): 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 4345 4391 4649 4666 4702  
 3887 3906 4598  
 draI(TTTAAA): 562 2480  
 draIII(CACNNNGTG): 290 1199 1629 4411  
 eaeI(YGGCCR): 290 1199  
 eagi(CGGCCG): 290 1199  
 earI(CTCTTC): 3008 4812  
 ecoO109I(RGGNCCY): 640 1623[dcn-] 1665 4998  
 ecoRI(GAATTC): 1  
 ecoRII(CCWGG): 501 524 1627 3157 3278 3291  
 ecoRY(GATATC): 911

FIG.-12K

SUBSTITUTE SHEET

37/55

fnuHHI(GCNGC): 204 207 479 1198 1201 1221 1384 1393 1472 1591 1594 1601 1615 1744 1747 1870  
 1951 2664 2686 2700 2769 2866 2919 3035 3053 3056 3174 3329 3472 3537 3540 3746  
 4074 4263 4413 4440 4535 4764  
 fnuHII(CGCG): 211 1203 1419 1429 1574 1600 1722 1819 2191 2292 2668 2688 2712 2730 2732 2835  
 3176 3757 4087 4580 4912  
 238 811 959 963 1866 1955 2033 2194 2805 4003 4184 4471  
 1541 1639 4243  
 fspI(TGCGCA): 471 526 1629 3143 3154 3606  
 fspI(TGCGCA): 471 526 1629 3143 3154 3606  
 haeI(WGGCCW): 153 1390 1829 1912 2632 2640 3004 3374  
 haeI(RGCGCY): 291 472 527 1200 1446 1630 2134 2334 2476 3144 3155 3173 3607 4065 4145 4412  
 haeIII(GGCC): 4999  
 hgaI(GACGC): 1425 1575 2189 2713 2836 3232 3810 4560  
 hgaI(GACGC): 1425 1575 2189 2713 2836 3232 3810 4560  
 hgiI(GGRC): 495 1139 1650 2946 3444 4605 4690  
 hgiI(GGRC): 495 1139 1650 2946 3444 4605 4690  
 hgiI(GGRC): 1474 2522 3971  
 hgiI(GGRC): 2556  
 hgiI(GGRC): 112 154 210 1391 1542 1604 1640 1830 1913 2633 2641 2667 2689 2711 2731  
 hgiI(GGRC): 2834 2864 3005 3038 3308 3375 3475 3649 3758 4151 4244 4581 4913  
 hgiI(GGRC): 112 154 210 1391 1542 1604 1640 1830 1913 2633 2641 2667 2689 2711 2731  
 hgiI(GGRC): 2834 2864 3005 3038 3308 3375 3475 3649 3758 4151 4244 4581 4913  
 hgiI(GGRC): 1206 4562  
 hgiI(GGRC): 1206 4562  
 hgiI(GGRC): 71  
 hgiI(GGRC): 505 685 901 1489[M.hphI-] 1710[M.hphI-] 2216 2412 2434 3030 3105 3501 4018  
 hgiI(GGRC): 1206  
 hpaI(GTAAAC): 1443 1469 1670 1850 1997 2257 2587 2717 2776 2810 3337 3484 3510 3700 4104 4138  
 hpaI(GTAAAC): 1443 1469 1670 1850 1997 2257 2587 2717 2776 2810 3337 3484 3510 3700 4104 4138  
 hpaI(GTAAAC): 4205 4315 4557  
 hphI(GGTGA): 380 561 575 1492 1713 2494 2740 2749 3874 4101 4497 4723 4738  
 mboI(GAAGA): 409 542 1181 1184 1786 2613 3009 3780[dam-] 3871[dam-] 4626 4704[dam-] 4813  
 5009  
 mboI(dam-)(GATC): 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 4345 4391 4649 4666 4702  
 mnlI(CCTC): 148 163 241 372 378 470 614 759 865 1136 1157 1413 1451 1478 1664 1978 2036  
 2092 2520 2727 2757 3019 3245 3302 3569 3969 4050 4180 4386 4997  
 msei(TTAA): 69 257 324 519 744 893 1207 1905 2127 2159 2273 2284 2296 2307 2324 2422 2693  
 2911 3836 3888 3893 3907 3960 4195 4234 4599 4971  
 mspi(CCGG): 1443 1469 1670 1850 1997 2257[M.bamHI-] 2587 2717[M.bamHI-] 2776 2810 3337 3484  
 3510 3700 4104 4138 4205 4315 4557  
 1541 1639 4243  
 msti(TGCGCA): 1468 2586  
 naei(GCCGGC): 1443 1669 1997 2775 2810 3509 4205 4556  
 nci(CCSGG): 2952  
 ndei(CATATG): 1387  
 nhei(GCTAGC): 1387

FIG.-12L

SUBSTITUTE SHEET

38/55

nlaII(CATG): 40 597 623 905 1176 1332 1436 1643 1777 2002 2066 2131 2766 2871 3131 3851 4342  
 4352 4430 4466 4859 4964  
 nlaIV(GGNCC): 550 641 1024 1339 1439 1474 1509 1623 1666 1945 2253 2522 2543 2555 2719 3160  
 3199 3971 4065 4106 4317 4907  
 natI(GCGCGCCG): 1198  
 natII(ATGCAT): 453  
 nspCIX(RCATGY): 1175 2001 2765 3130  
 p1MI(CCANNNNTGG): 14 1500 1549  
 pleI(GAGTC): 505 685 2412 2434 3030 3501 4018  
 ppMI(RGWCCY): 640 1623 1665  
 pti(CTGCAG): 4264[M.HI-]  
 pui(CGATCG): 4390  
 pvuII(CAGCTG): 270 650 733  
 xbaI(GTAC): 159 342 627 804 1054 2937 4502  
 xba3AI(GATC): 139 851 1095 1340 1646 1853 2254 2720 3697 3772 3783 3791 3869 3881 3986 4327  
 4345 4391 4649 4666 4702  
 xba96I(GGNCC): 641 1024 1445[M.haeIII-] 1624[dcn-] 1666 1945 2134[M.haeIII-] 2476[M.haeIII-]  
 4065[M.haeIII-] 4144[M.haeIII-] 4161 4383 4999[M.haeIII-]  
 4501  
 xbaI(AGTACT): 1443 1669 1997 2775 2810 3509 4205 4556  
 xcrFI(CCSGG): 501 524 1627 3157 3278 3291  
 xcrFI[dcn-](CCWGG): 175 237 416 1252 1362 1606 1858 1867 1954 2032 2095 2806 2922 2977 2998 3218  
 xfaNI(GCATC): 4270 4480 4710  
 217  
 xnaBI(TACGTA): 338  
 xpeI(ACTAGT): 2275 4825  
 ssPI(AATATT): 526[dcn-]  
 xtuI(AGGCTT): 637 1554  
 styI(CCWVGG): 1453 2518 3230 4674  
 taqI(TCGA): 211 1203 1419 1429 1574 1600 1722 1819 2191 2292 2668 2712 2730 2732 2835  
 :haI(CGCG): 3176 3757 4087 4580 4912  
 2874  
 tthIII(GACNNNGTC): 368  
 xbaI(TCTAGA): 850 1094 1339 1852 2253 2719 3771 3782 3868 3880 4648 4665  
 xhoII(RGATCY): 290 1199  
 xmaIII(CGCGCG): 2216 4618  
 xmnI(GAANNNTTC):  
 not found:

aflII(CTTAAG), apaI(GGGGCC), asp718(GGTACC), asuII(TTCGAA), bglII(AGATCT), bspMI(ACCTGC), bssHI(GCGCGC),  
 bstBI(TTCGAA), bsteII(GGTNACC), bstXI(CCANNNNTGG), bsu36I(CCTNAGG), clai(ATCGAT), eco8I(CCTNAGG), ecoNI(CCTNNNNNAGG),  
 espi(GGTACC), kpnI(AGCGGT), mluI(ACGGGT), mstII(CCTNAGG), nari(GGCGCC), ncoI(CCATGG), nruI(TCGCA), paeR7I(CTCGAG),  
 rsrII(CGGWCCG), sacI(GAGCTC), sacII(CGCGG), sali(GTCGAC), sfii(GGCCNNNGGCC), smaI(CCCGGG), sphi(GCATGC),  
 sstI(GAGCTC), xhoI(CTCGAG), xmaI(CCCGGG)

FIG.-12M

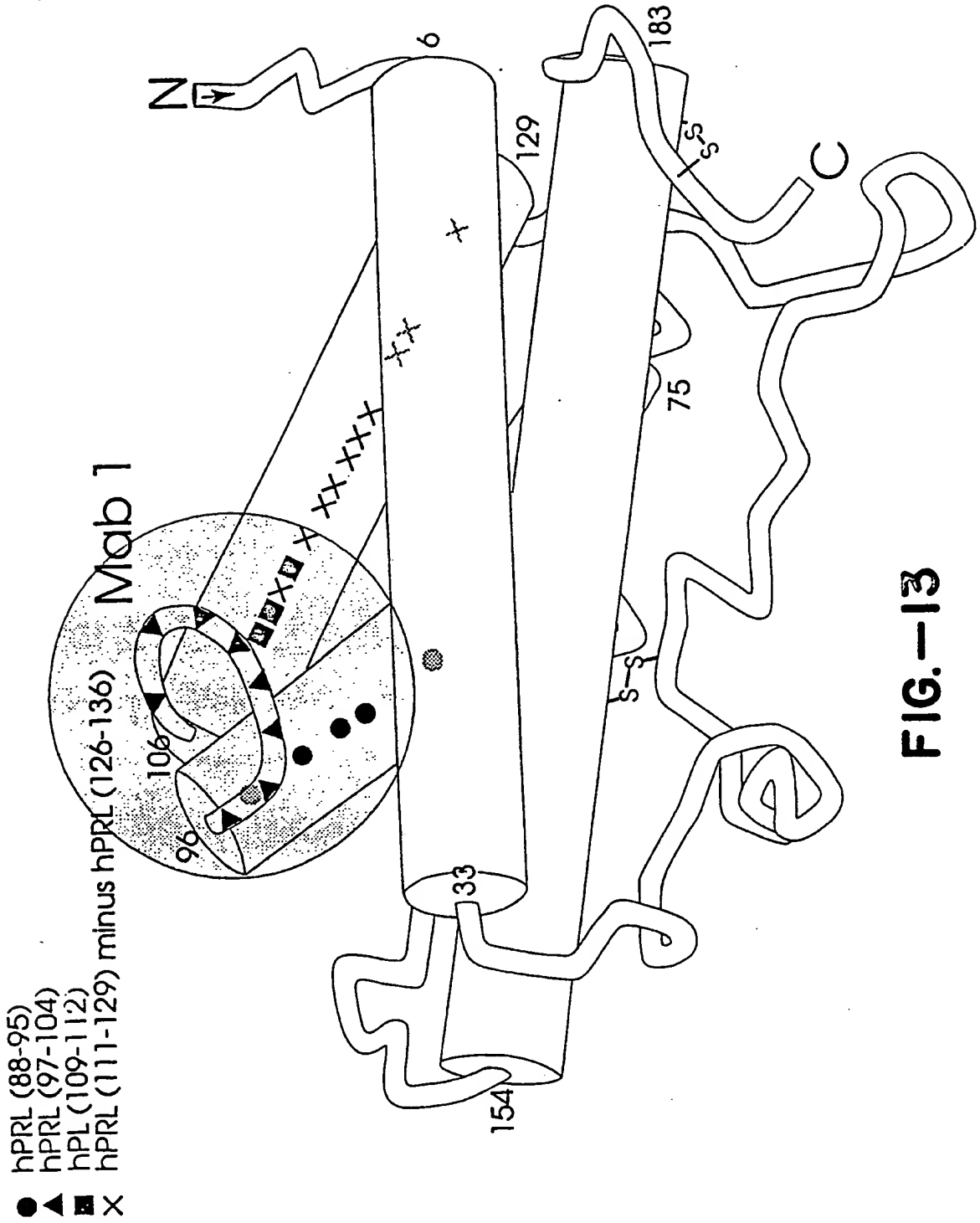
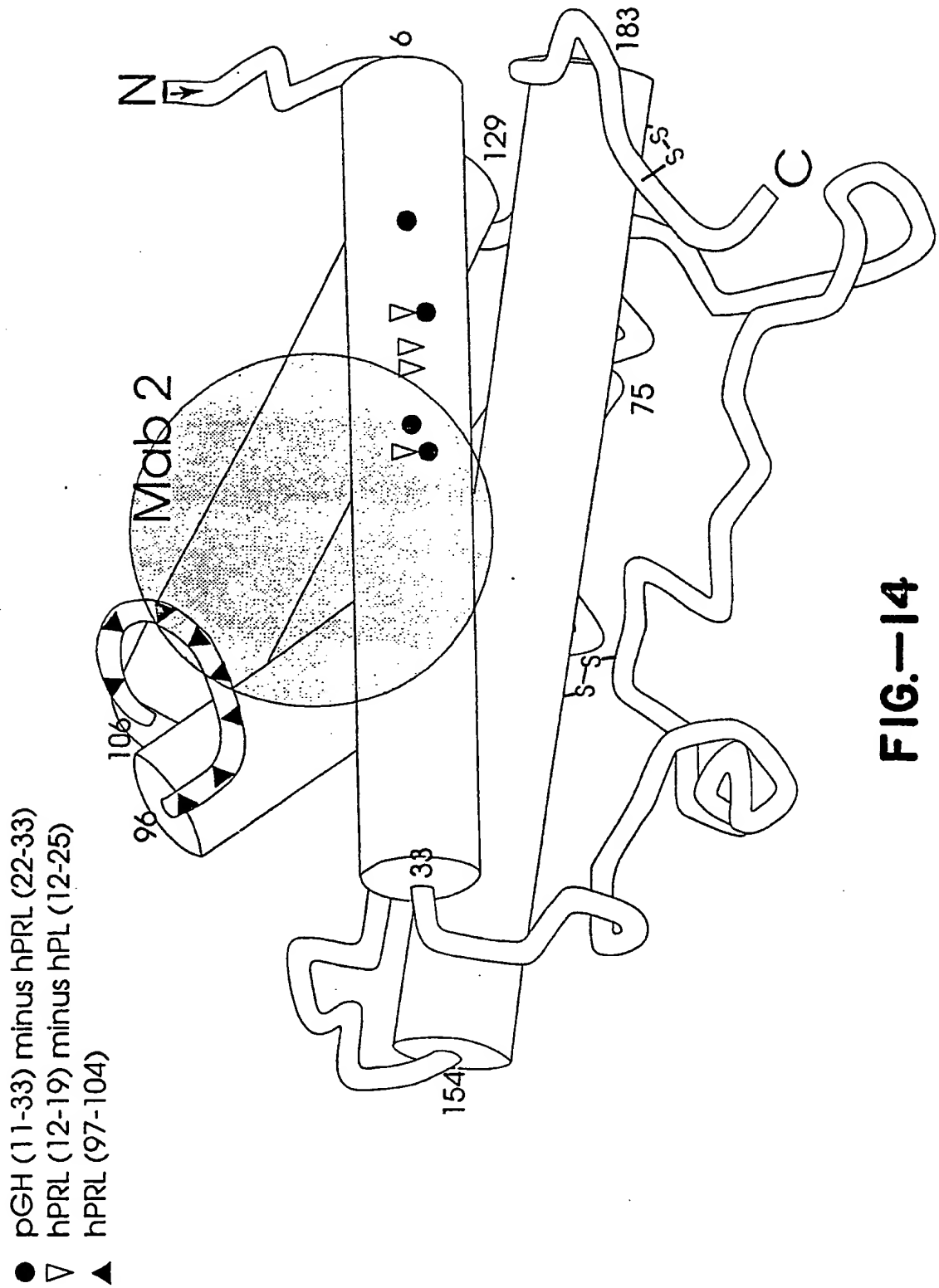


FIG.-13

40/55



**FIG. 14**

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- pGH (11-33) minus hPRL (22-33)
- ▲ hPL (12-25) minus hPRL (22-33)
- ▽ hPRL (12-79)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)

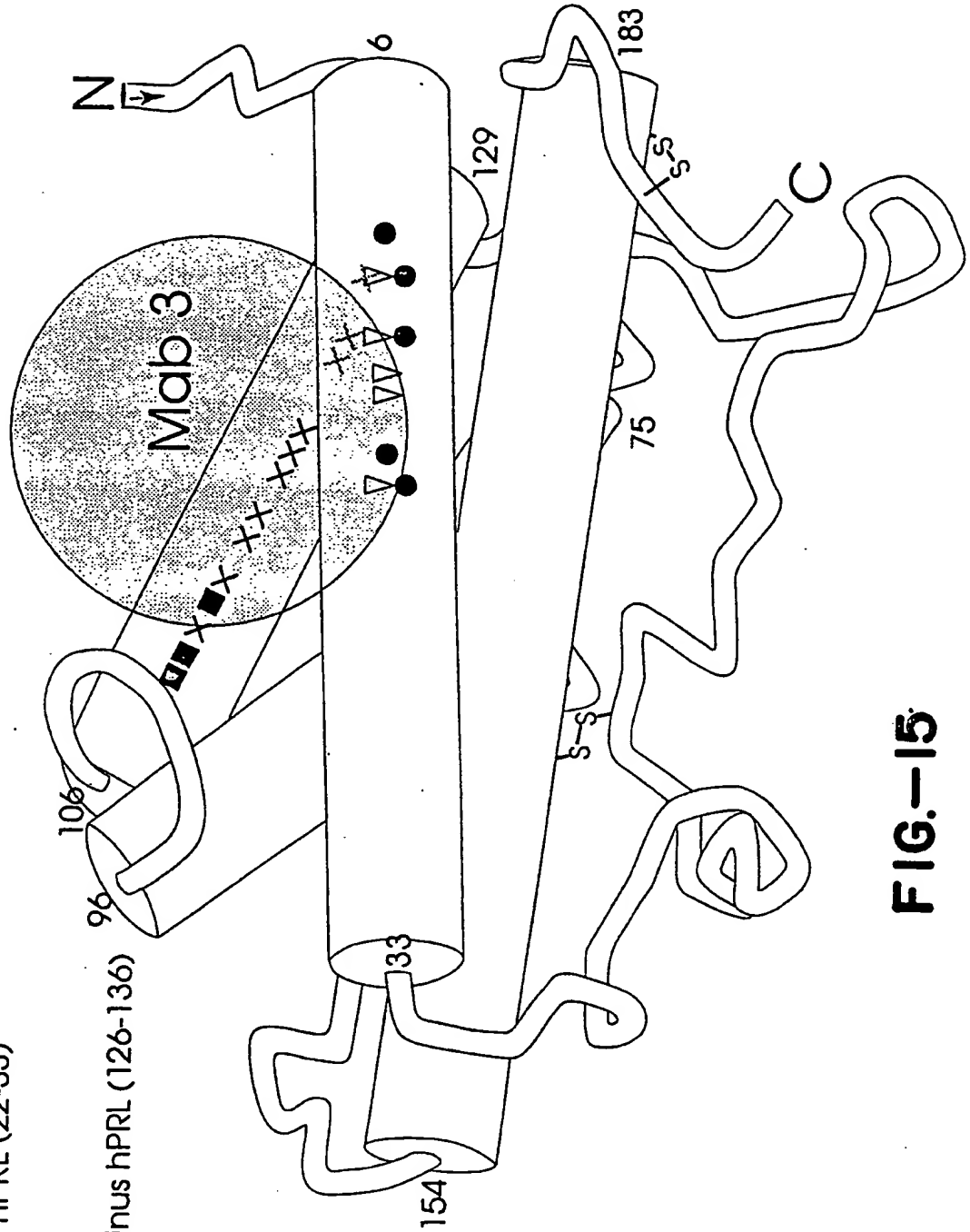


FIG.-15



42/55

- ▲ hPL (12-25) minus pGH (11-33)
- ▽ hPRL (12-19) minus pGH (11-33)
- hPL (109-112)
- × hPRL (111-129) minus hPRL (126-136)

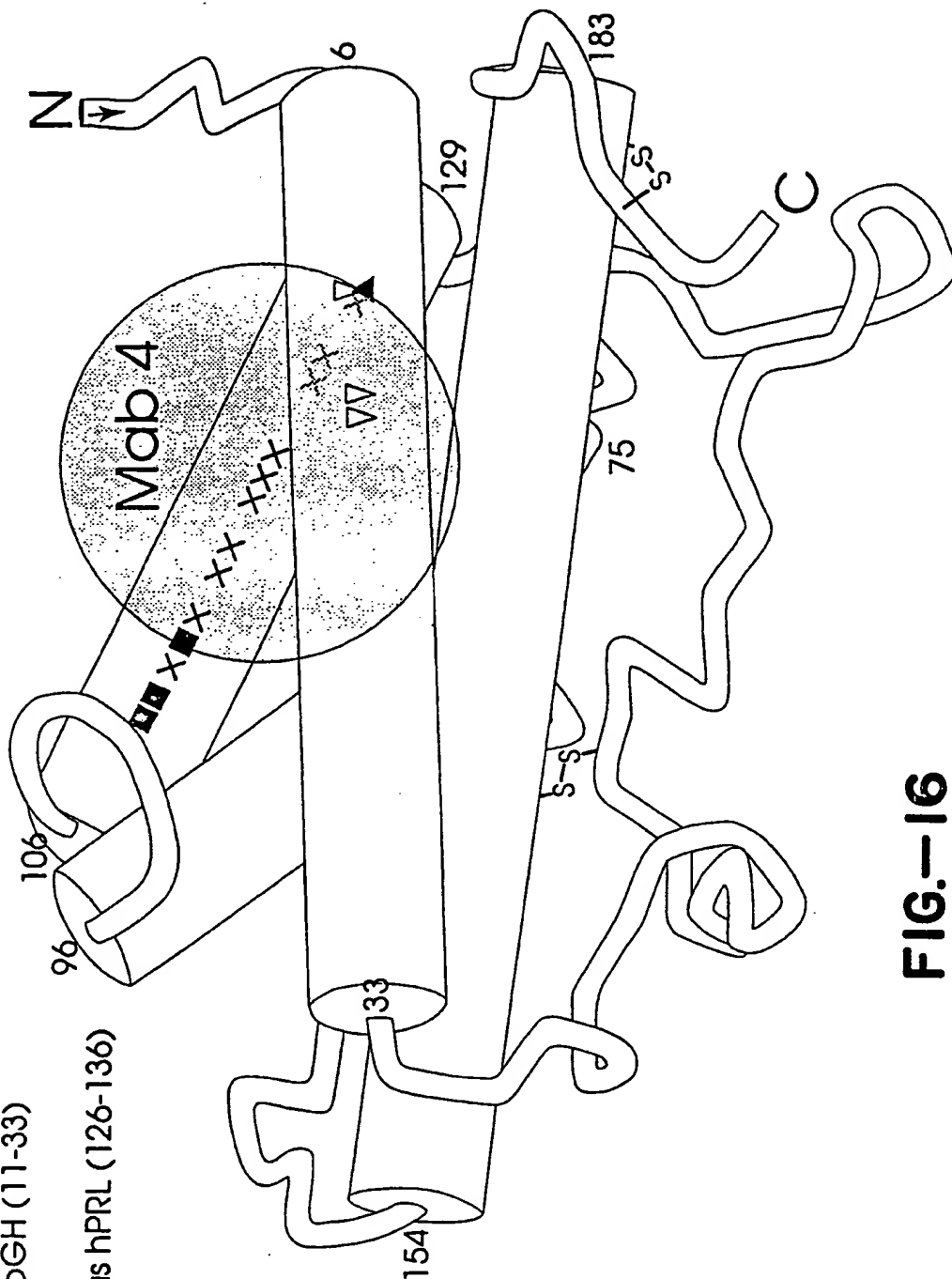


FIG.-16

43/55

- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)
- C182A

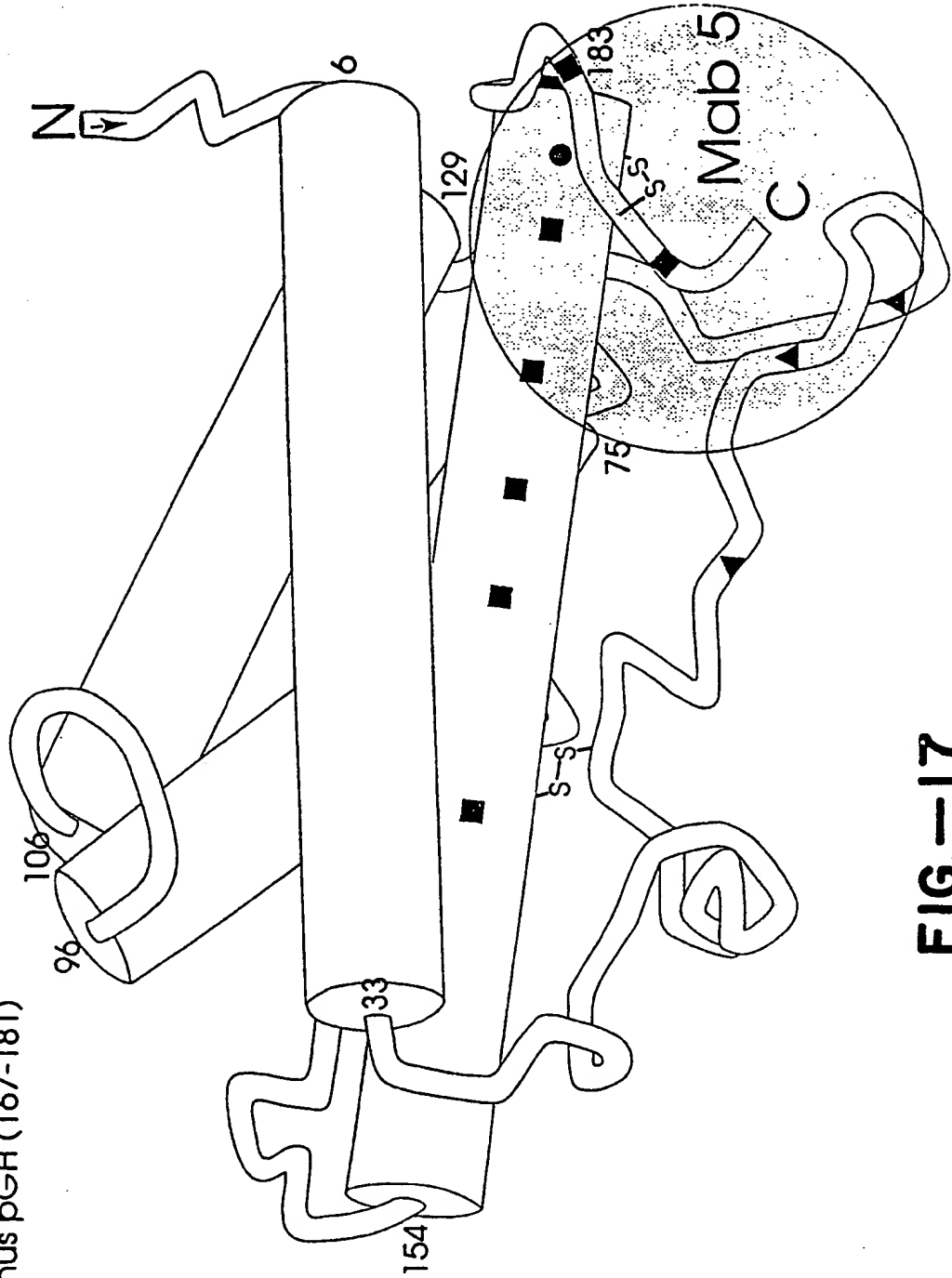


FIG.—17

44/55

- ▲ pGH (57-73) minus hPRL (54-74)
- pGH (164-190) minus pGH (167-181)

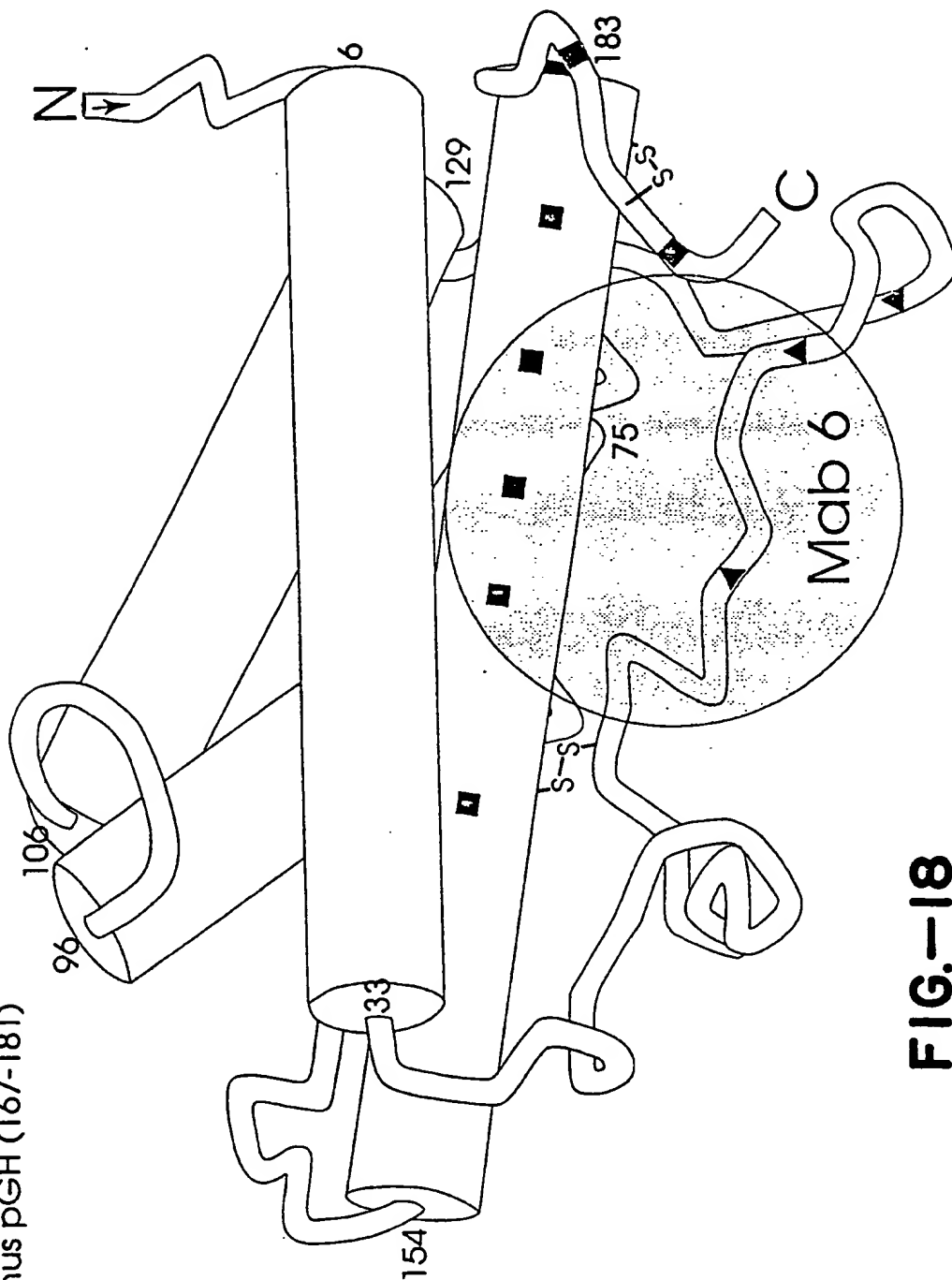
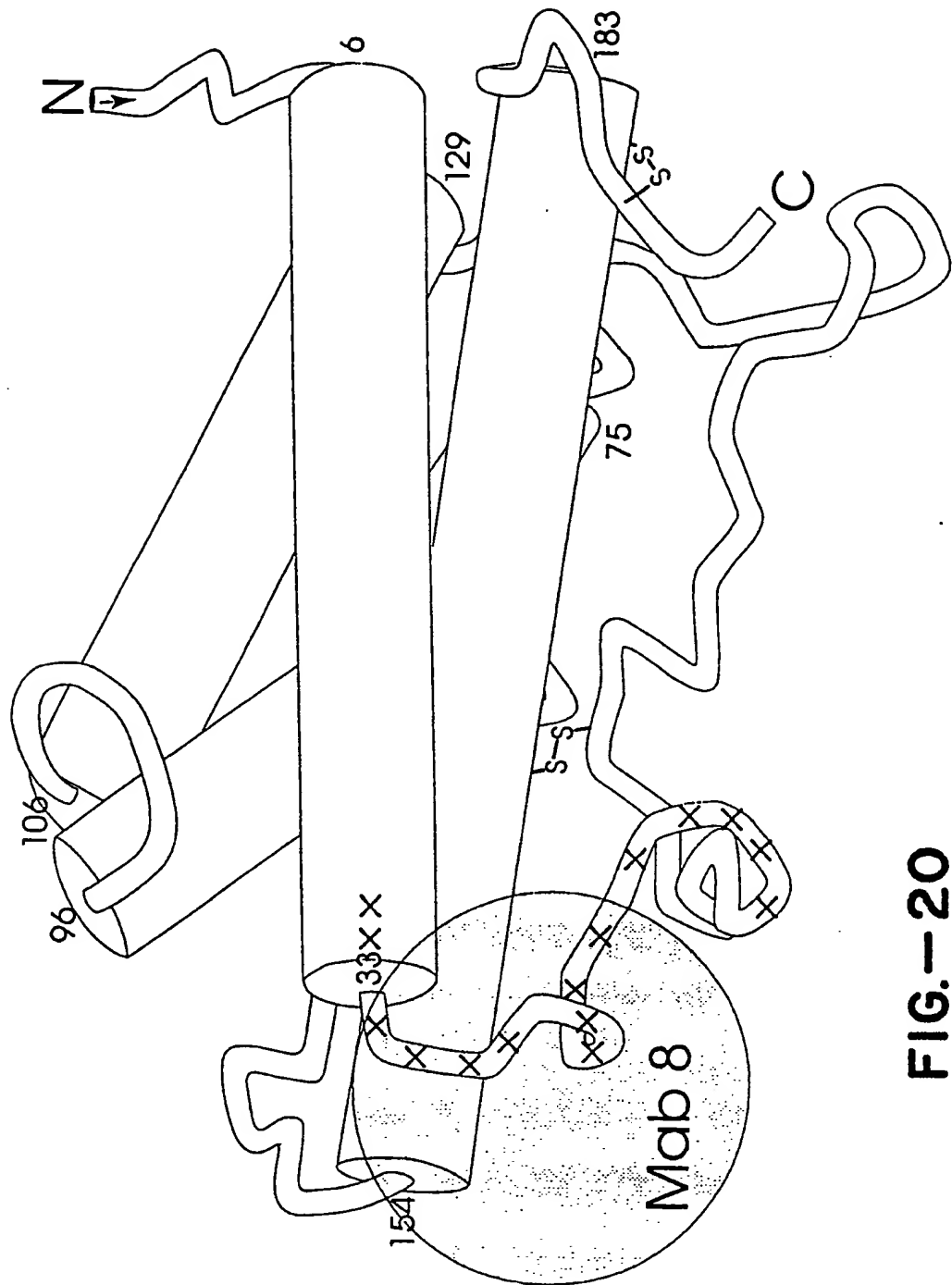


FIG.-18



46/55



**FIG. -20**

X Deletion (32-46)

**SUBSTITUTE SHEET**

47/55

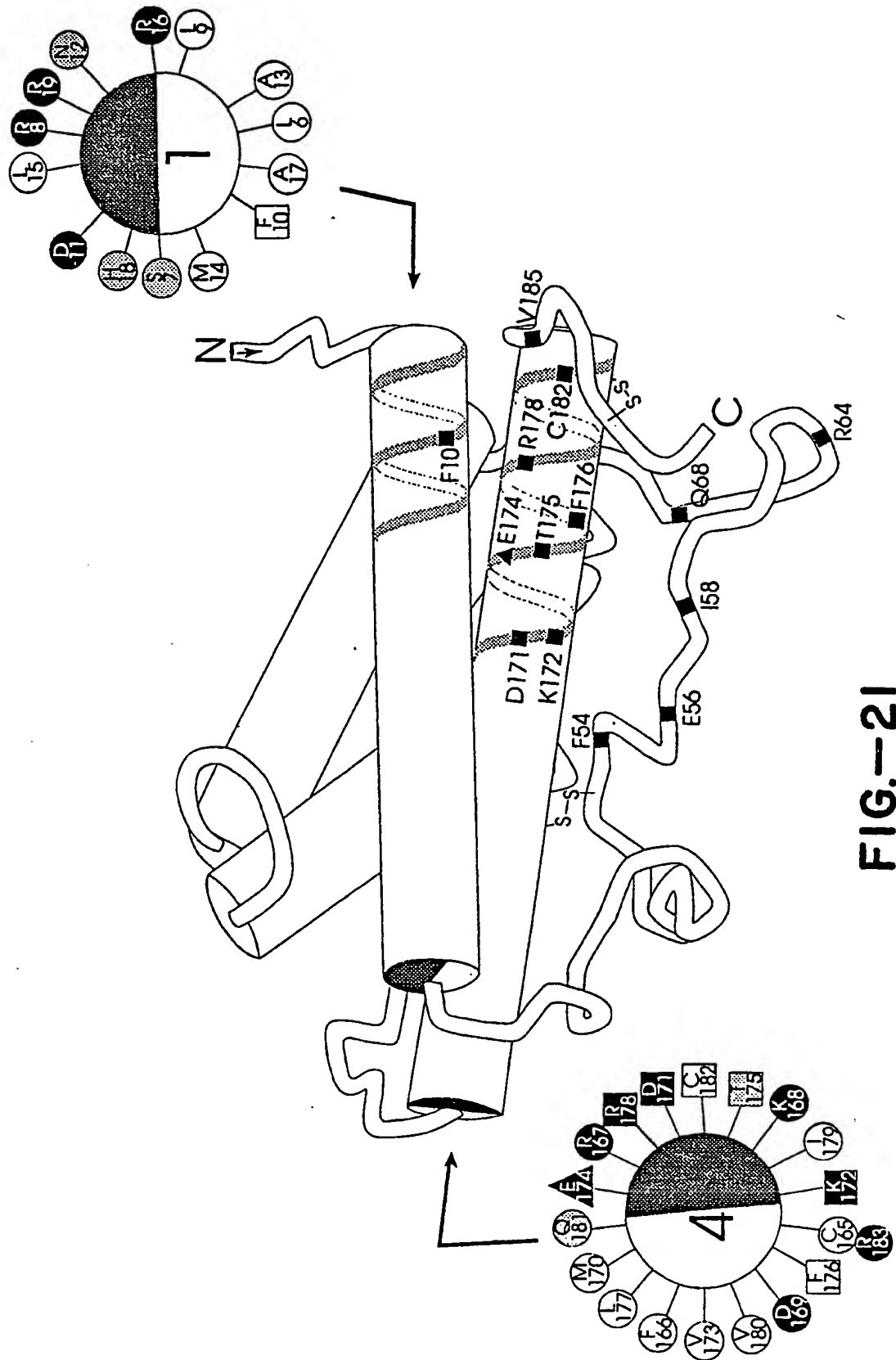


FIG.-21

48/55

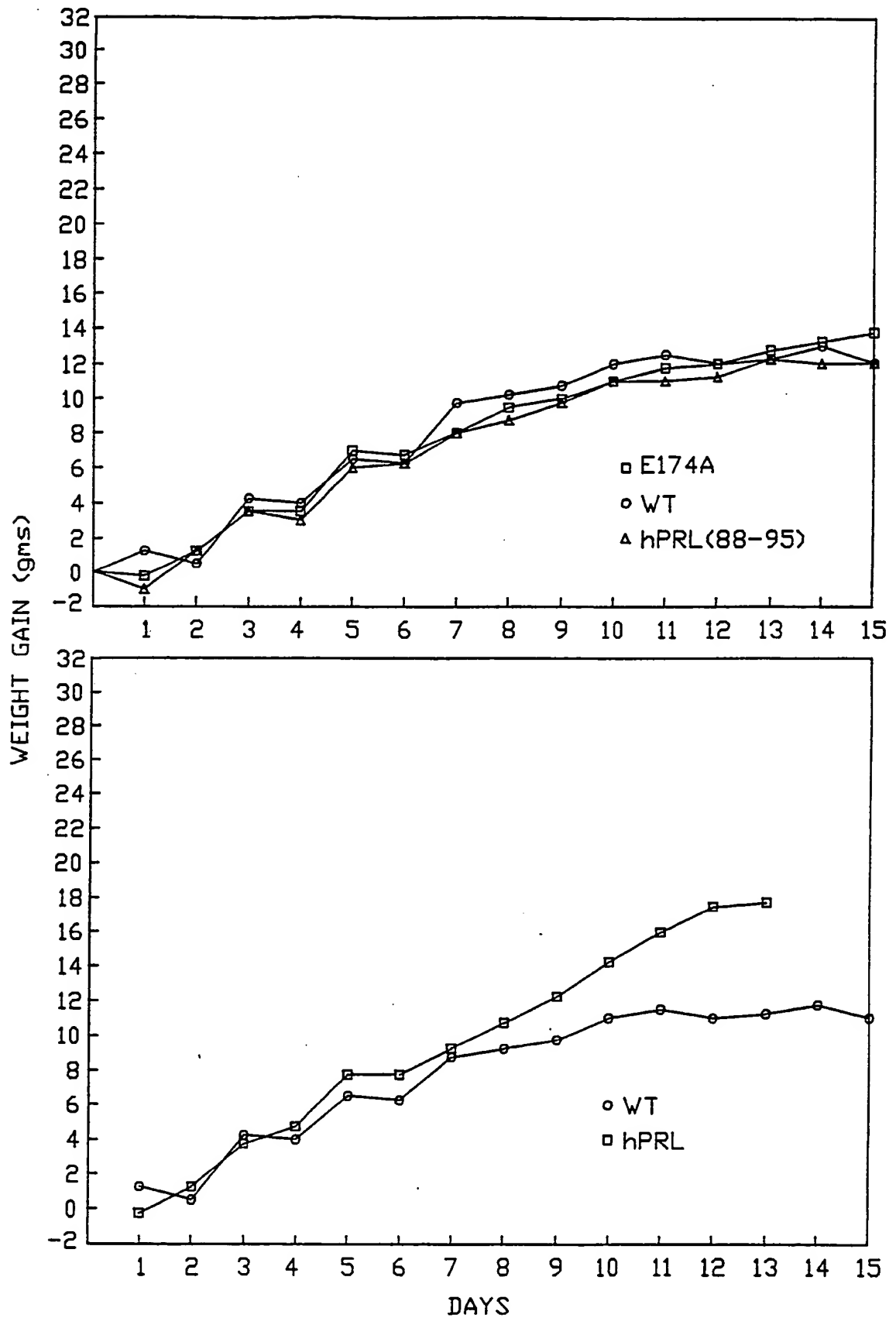


FIG.-22

SUBSTITUTE SHEET

## GH ANALOG BIO POTENCY IN RATS AFTER 8 DAYS OF TREATMENT

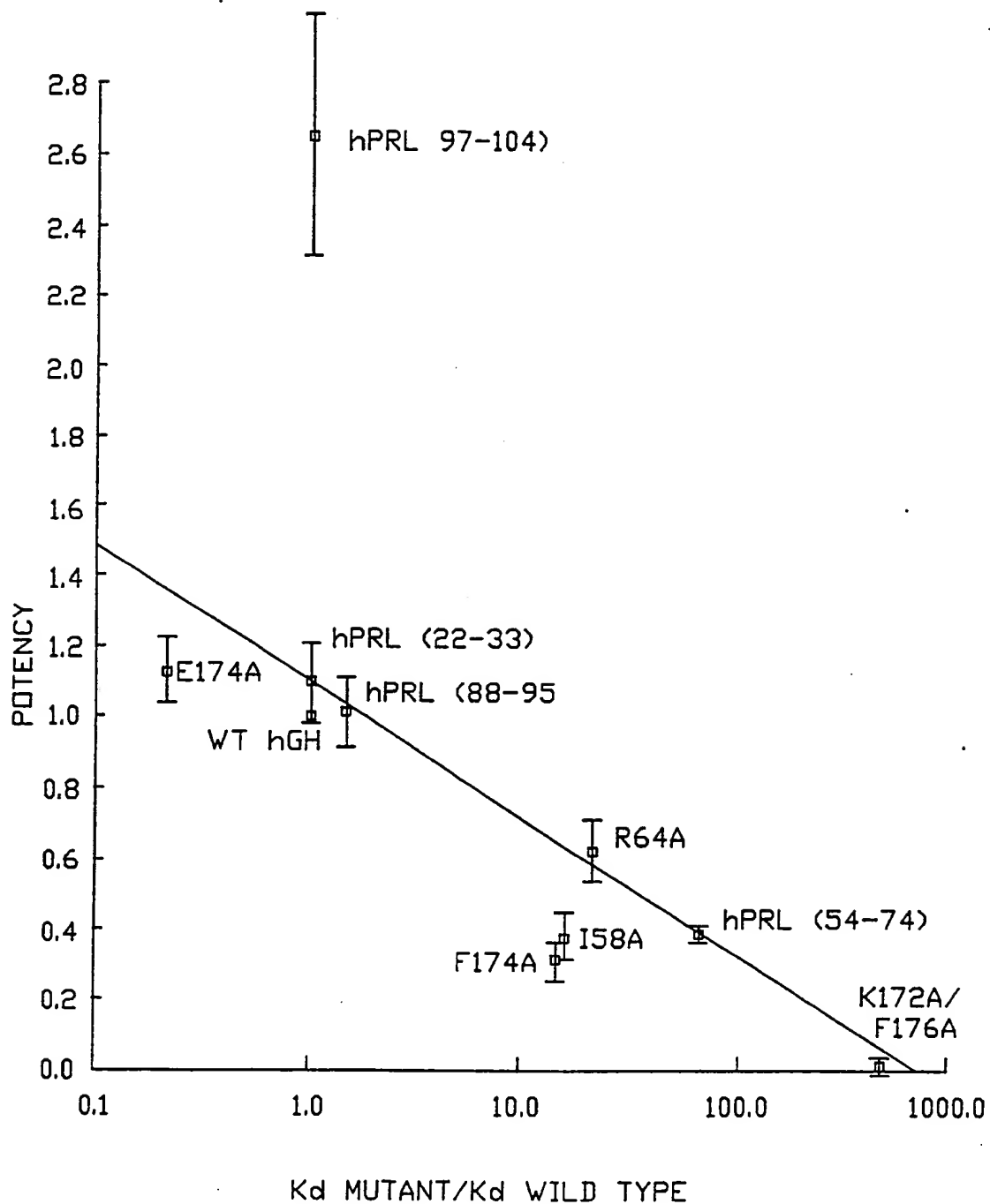


FIG.—23

SUBSTITUTE SHEET



50/55

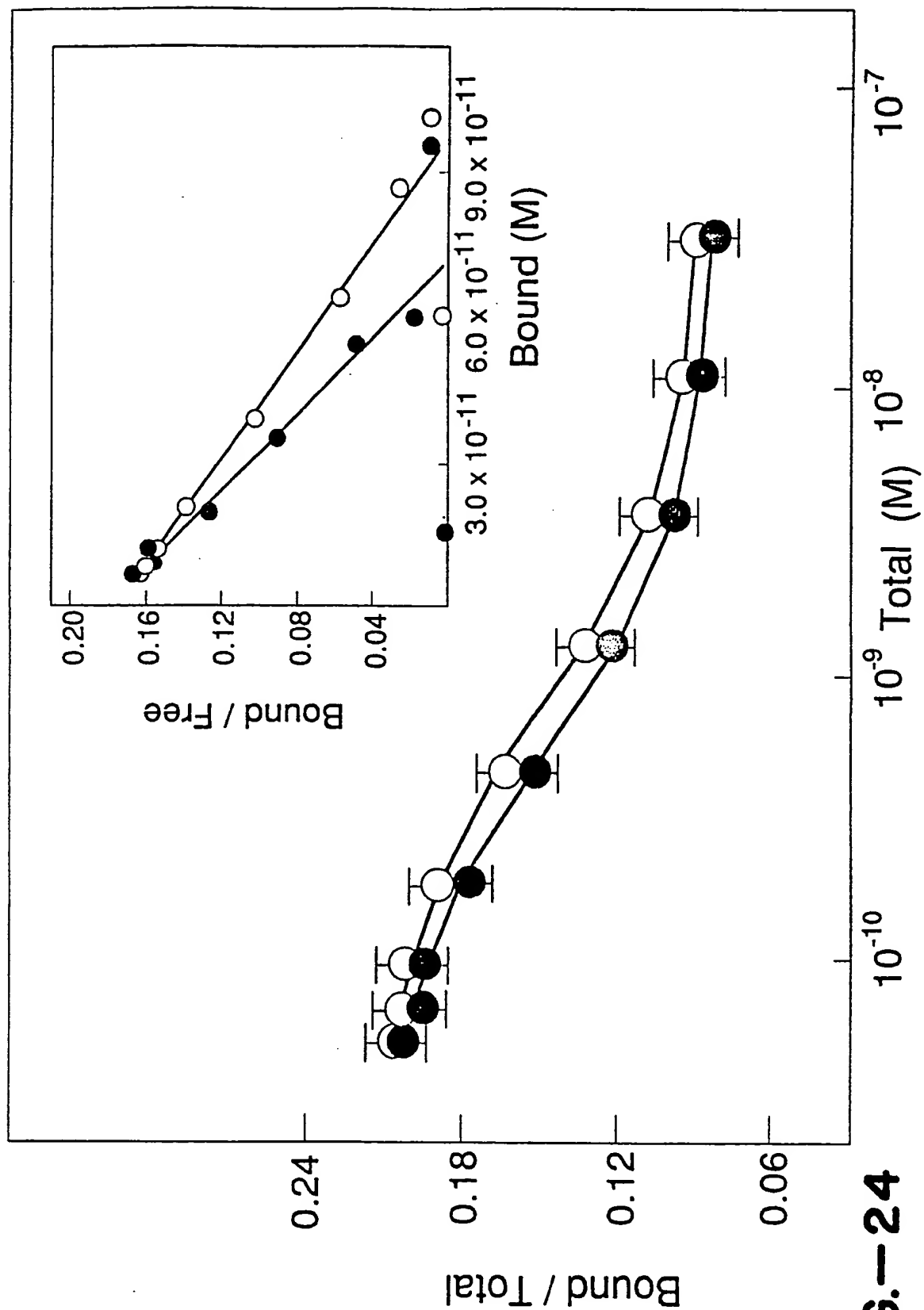


FIG.—24

SUBSTITUTE SHEET

## Binding Determinants for hGHR

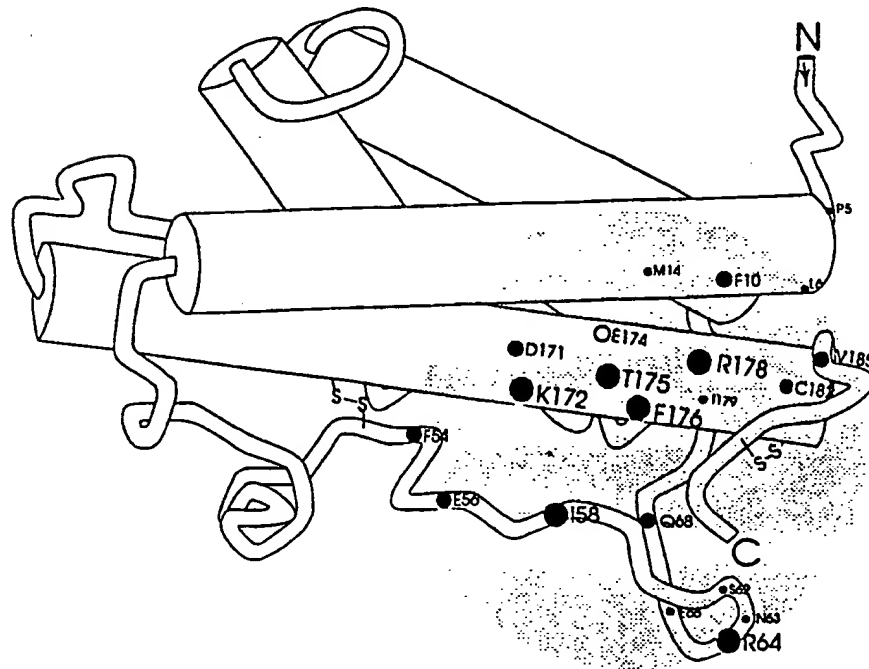


FIG.—25A

## Binding Determinants for hPRLr

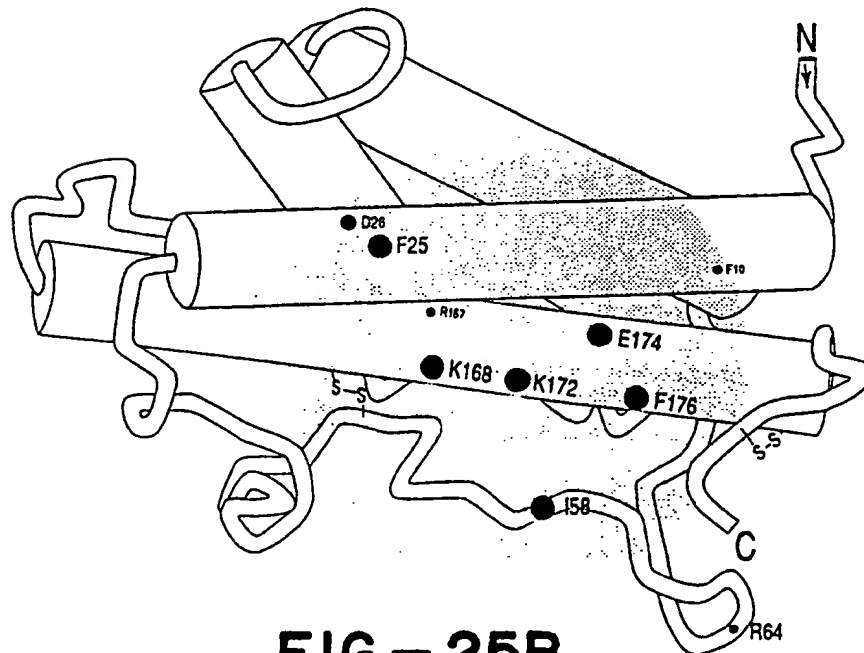


FIG.—25B

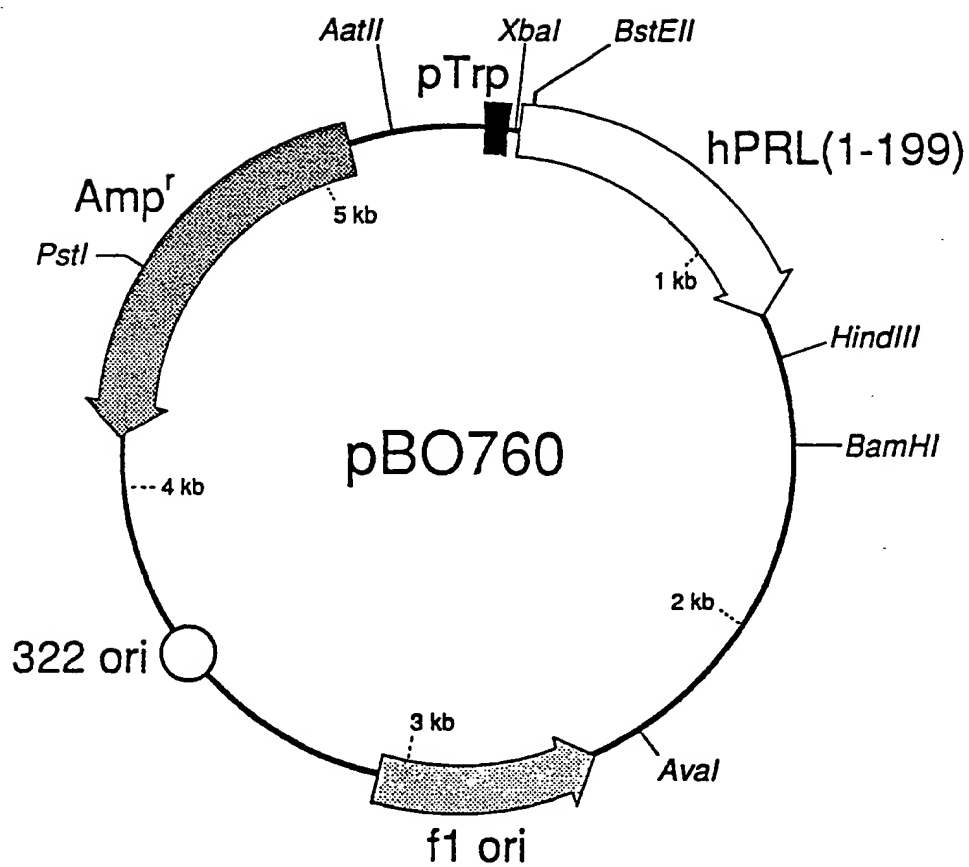


FIG.—26

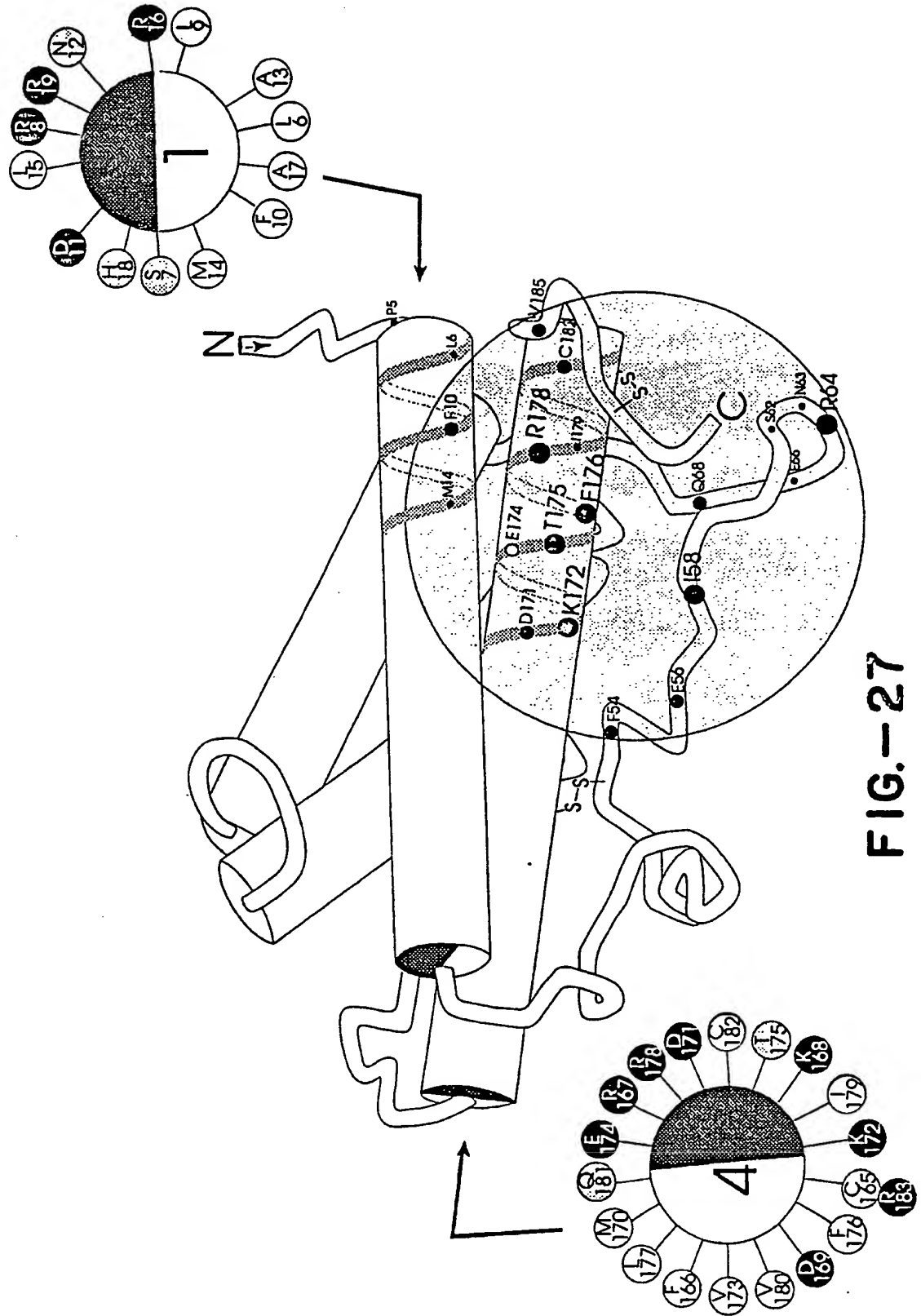
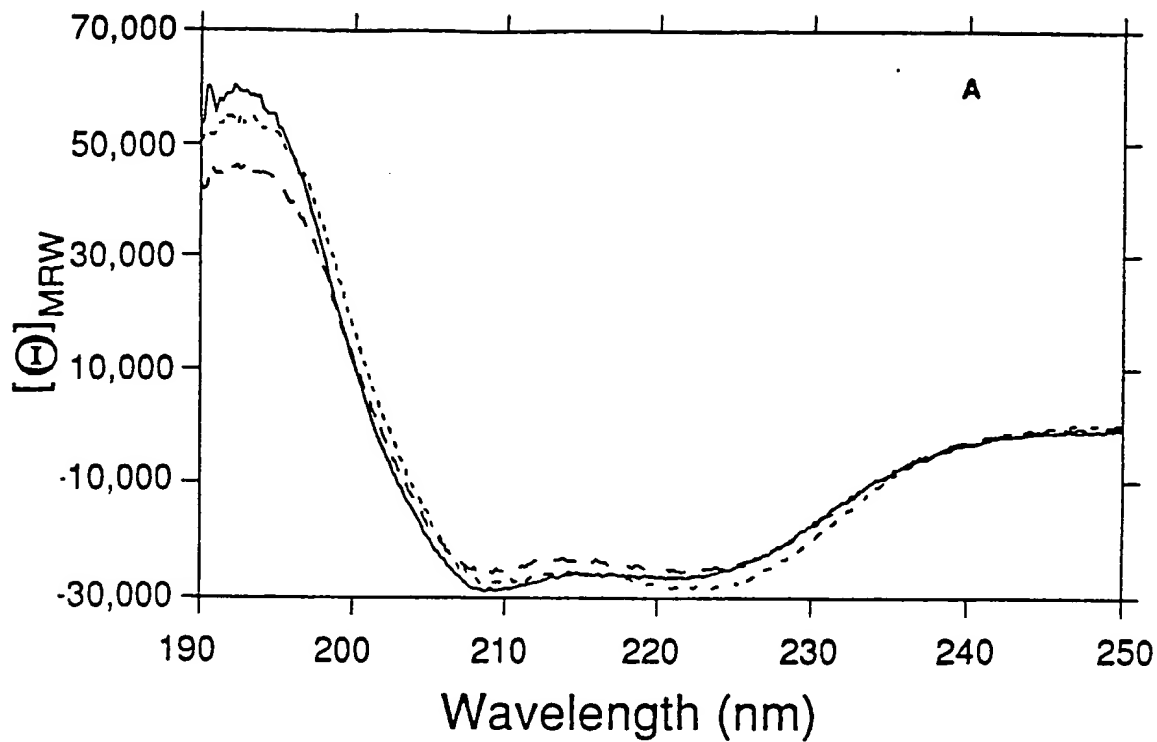
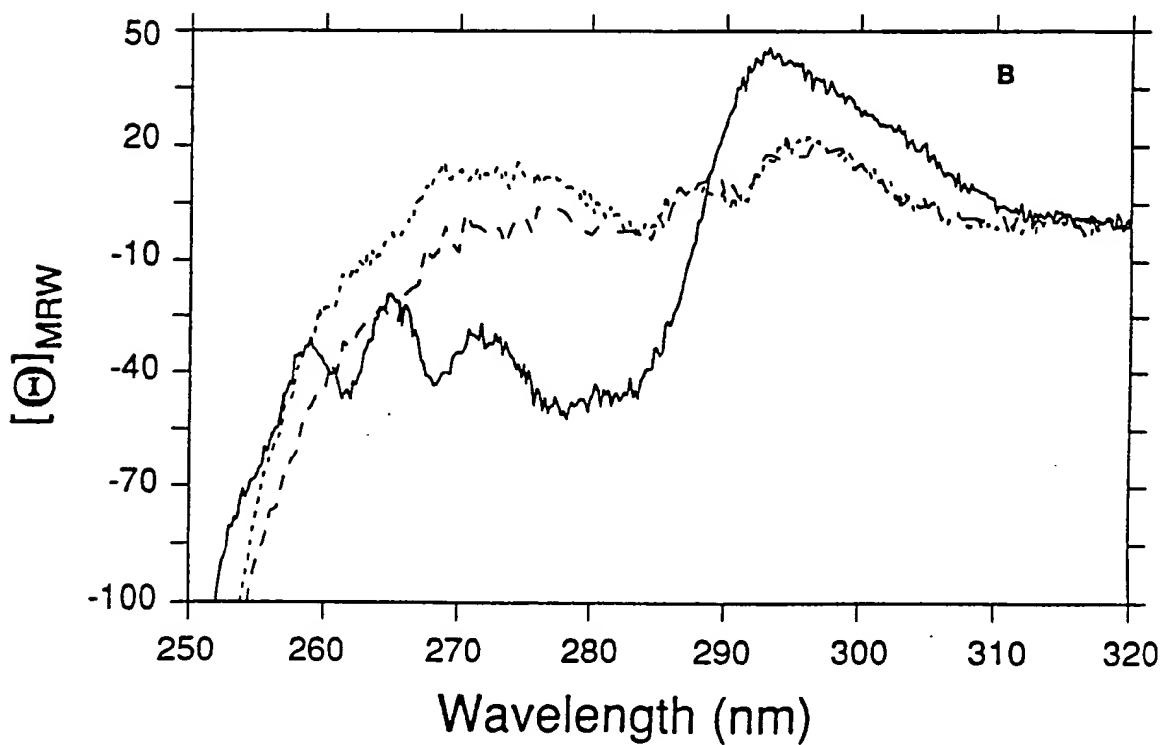


FIG.-27

**FIG.—28A****FIG.—28B**

55/55

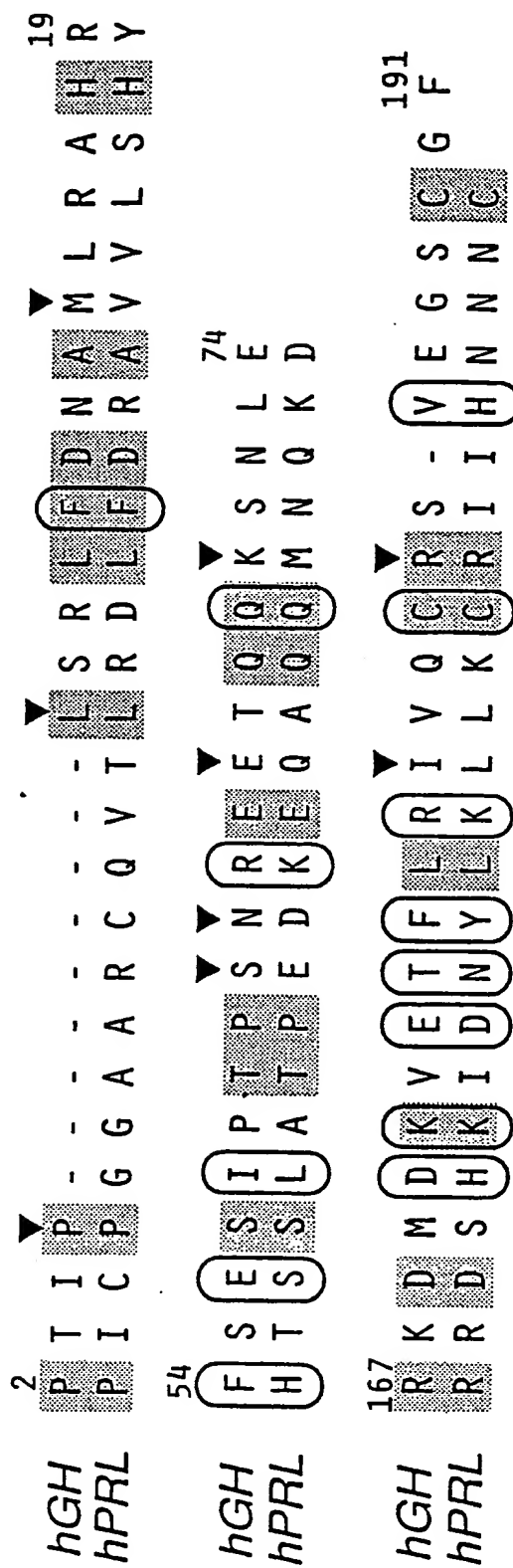


FIG.-29

SUBSTITUTE SHEET

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/04778

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): G01N 33/53, 31/00, 33/543, 33/567, 33/566 U.S. Cl: 435/7; 436/501, 504, 518, 548		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
U.S.	435/7; 436/501, 504, 518, 548; 935/79, 81	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
CHEMICAL ABSTRACTS SERVICE ONLINE, BIOSIS PREVIEWS, AUTOMATED PATENT SYSTEM		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>9</sup>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X <sub>2</sub> P	Science, Volume 244, issued 1989, CUNNINGHAM, B.C., ET AL, "High Resolution Epitope Mapping of hGH-Receptor Interactions by Alanine-Scanning Mutagenesis", 1081-1085.	1-13, 16-31
X,P	Science, Volume 243, issued 1989, CUNNINGHAM, B.C., ET AL, "Receptor and Antibody Epitopes in Human Growth Hormone Identified by Homolog-Scanning Mutagenesis", 1330-1336.	1-13, 16-31
A	Biochem. Biophys. Res. Commun., Volume 135, issued 1986, SOUROUTON, M.C., ET AL, "Localization of a Highly Immunogenic Region on the Acetylcholine Receptor Alpha-Subunit", 82-89.	1-13, 16-31
<p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
02 February 1990	05 MAR 1990	
International Searching Authority	Signature of Authorized Officer	
ISA/US	KAREN I. KRUPEN	

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Endocrinol., Volume 121, issued 1987, WERTHER ET AL, "Localization and Characterization of Insulin Receptors in Rat Brain and Pituitary Gland Using In-Vitro Autoradiography and Computerized Densitometry, 1562-1570.	1-13,16-31
A	Endocrinology, Volume 107, issued 1980 MILLS, T.B. ET AL, "Fragments of human growth hormone produced by digestion with thrombin: chemistry and biological properties", 391-399 (See Abstract, 143544)	1-13,16-31
A	Chemical Abstracts, Volume 108, no. 11, issued 1988, (Columbus, Ohio, U.S.A) B. C. Cunningham, "Improvement in the alkaline stability of subtilisin using an efficient random mutagenesis and screening procedure", Abstract.	1-13,16-31



## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE<sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . . . because they relate to subject matter<sup>12</sup> not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . . . because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out<sup>13</sup>, specifically:

3. ☐ Claim numbers . . . . . because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING<sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

(See Attachment).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: 1-13 and 16-31

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

ATTACHMENT TO PCT/ISA/210

Part IV. Before #1, Observations

- I. Claims 1-13 and 16-31 are drawn to a method for identifying unknown active domains in the amino acid sequence of polypeptides classified in class 436, subclass 501.
- II. Claims 14, 15 and 32-64 are drawn to a method of forming a growth hormone variant and the growth hormone variants produced classified in class 530, subclass 350.
- III. Claims 65-79 are drawn to human prolactin hormone variants classified in class 530, subclass 399.
- IV. Claims 80-83 are drawn to human placental lactogen variants classified in class 530, subclass 399.
- V. Claims 84-86 are drawn to DNA sequences and expression vectors and hosts classified in class 536, subclass 27.